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THE

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AND

## Animal Husbandry

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# The Indian Journal of Veterinary Science and Animal Husbandry

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## ORIGINAL ARTICLES

### HEREDITY AND DISEASE RESISTANCE

BY

PROF. F. A. E. CREW, M.D., D. Sc., PH.D.

*Director, Imperial Bureau of Animal Genetics, University of Edinburgh.*

(Received for publication on 10th January, 1938)

OBSERVATION and experiment have shown clearly and conclusively that constant and true-breeding differences in resistance to particular diseases distinguish the species and that in all probability such differences are paralleled by differences which distinguish individuals of one and the same species. This is as would be expected by the geneticist. It is indeed safe to assert that such resistance and susceptibility are genetic, in whole or in part, and that natural selection would tend to eliminate the susceptible and produce a population more resistant than one which had not been subjected to such natural selection.

Granting this it follows that the genetic improvement of an animal population is stopped by any measures that prevent selection. Prophylaxis and therapy must necessarily prevent genetic improvement in respect of resistance because the less resistant are saved to reproduce.

There can be little doubt that the resistance of an individual host to a given infection is determined largely by genetic factors but the value and the nature of these factors have not yet been disclosed. We do not know how these factors produce their effects, whether they are specific or non-specific, and we do not know whether such resistance is uni- or multi-factor in its origin. Experiment has shown that between strains of the same species there are differences in mortality from specific infections which are to be referred to genetic differences between the strains. Selective breeding within the strain on the other hand has yielded results which lack uniformity. If resistance

NOTE.—It should be explained that the above note by Prof. F. A. E. Crew was not written for publication but for the purpose of taking part at a discussion on "Heredity and Disease Resistance," held at the Jubilee Session of the Indian Science Congress, Calcutta, 1938. In view of the importance of the subject his permission has, however, been obtained to publish it as it stands. The feasibility of breeding disease-resistant strains of domesticated animals is a matter regarding which there has been considerable difference of opinion and the clear expression of opinion contained in this note, by such an authority as Professor Crew, should help to clarify the position.—[Ed.]

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*Heredity and Disease Resistance*

were purely genetic it would be expected that increasing and continued selection would yield an increasing uniformity in genetic constitution and increasing uniformity in respect of resistance. But this is not always apparent and it is as yet quite impossible to state that by such means a fixed degree of resistance could be realised. It is not even clear that the manifestation of these genetic qualities is regular and orderly; resistance would seem in general to be an irregular dominant.

The possibility of breeding strains of animals of economic importance with fairly fixed high grades of resistance, at least to a particular agent, seems to be an eminently reasonable proposition. But it has to be confessed that in many of the experiments on the results of which this opinion is based the possibility that environmental factors have been operating has not been definitely excluded.

To design and to complete an experiment of this magnitude is indeed most difficult; to satisfy the bacteriologist, the geneticist and all the rest who have the right to comment and to criticise, is well-nigh impossible at the present time because of the insufficiency of our knowledge. To the breeder of animals of economic importance the difficulties are almost overwhelming. He must work with animals known to be free from the particular infection under consideration and he must keep them free at least until their progeny have been weaned. To incorporate resistance into a group he must either demonstrate that it is already possessed by certain of the individuals or else introduce it by crossing. Selection of the kind required adds to the number of characters already being selected for and therefore complicates the breeding programme. And if and when he reaches his objective, mutation of the pathogen can upset all his plans and force him to begin all over again.

It seems probable that where adequate methods of prophylaxis or therapy are known and readily applicable they will, in general, be preferable economically to any attempt to breed for resistance. From the nature of the case the number of diseases to be selected against in any animal stock must necessarily be limited and the genetic method should be reserved for those which cannot readily be controlled by other means. It seems rather hopeless to breed for resistance save only on a large scale with animals producing numerous progeny rapidly. The plan demands large numbers and large scale elimination. This means, under most conditions, that the genetic method can be employed only in the case of animals, individually of but little commercial value.

## A FORM OF VERMINOUS OPHTHALMIA IN EQUINES\*

BY

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Mukteswar

(Received for publication on 9th January, 1938)

(With plates I and II)

In certain parts of India, an obscure disease affecting the eyes of horses has been known under the name "Periodic Ophthalmia" for many years. The disease occurs in the horse breeding establishments at Montgomery and Probbynabad in the Punjab and during recent years a good many cases of eye affection have been met with. Work on the investigation of this condition was undertaken at this Institute in the year 1934 and the following information was elicited on enquiry.

Although the diagnosis "Periodic Ophthalmia" has been in common use for many years in the affected farms and in Army Veterinary Service, the disease is not by any means periodic, but is rather of a recurrent nature.

The disease has been established in the stud farms at Coleyana and Probbynabad for several years and probably considerably longer and one or two animals are often becoming affected. The aetiology of this condition has for many years been the cause of much controversy, trauma, dietetic deficiency and bacteria being falsely incriminated.

### SYMPTOMS

*Initial stage.*—An attack of this disease is ushered in more or less suddenly, i.e., it may be discovered in the morning, though nothing was noticed on the previous night. There is marked local irritation, photo-phobia and lachrymation; there is acute pain present, the eyelids are closed and the animal objects to manipulation of the eye. On examination there is marked general conjunctivitis and redness associated with ciliary congestion. The cornea may show slight opacity at its margin and this opacity may later spread over the cornea. In some cases small blood vessels may be seen invading the cornea. The pupil remains contracted. The iris may appear swollen and rigid and loses its lustre, assuming a greyish or lighter colour than normal owing to congestion and exudation. Sometimes the iris appears adherent to the posterior surface of the cornea. In some cases the aqueous humour becomes turbid and occasionally there is a blood-stained deposit in it. The colour of the posterior chamber varies from greenish to yellowish green.

\* Paper read at the Indian Science Congress, Calcutta, 1938.

The intraocular pressure is often increased. These symptoms last for ten to fourteen days and then clear up rapidly. When these symptoms have cleared up, it may be difficult to detect any difference between the affected and unaffected eyes.

*Intermediary stage.*—When the disease recurs, the same eye or its fellow may be affected. Relapse occurs at intervals varying from a week to six months. The symptoms exhibited in later attacks may not be so acute as those in the initial attack, the lens gradually becomes more and more opaque. Between two attacks, at a stage when the opacity of the lens is not very appreciable, certain characteristic lesions such as small streaks or spots may be noticed on the lens-capsule and their lesions are indicative of chronic eye trouble.

*Last stage.*—After two or three attacks, the affected eyeball becomes shrunken and appears to be smaller than the unaffected one. There is marked wrinkling of the upper eyelid and in most cases the *membrana nictitans* protrudes across the eyeball. In such chronic cases, the pupil is widely dilated and the lens is opaque. A few cases have been seen when the pupil was contracted and the iris was apparently closely adherent to the lens.

As a rule apparent recoveries, followed by relapses, occur, and three or four such recurrent attacks cause permanent and complete blindness in the affected animals.

It is only in about 4 per cent of cases that both eyes are affected simultaneously. Usually one eye is affected at a time and relapses occur after apparent recovery. The other eye may be affected either in the intervals or after the first eye has become blind. It also happens at times that one eye remains unaffected throughout the course of the disease. In one interesting case, one particular eye was repeatedly affected, the duration of the affection extending over a long period, culminating in blindness in the one eye. This eyeball was then removed for experimental purposes. Ten months after this removal, the remaining eye which had remained normal and unaffected, became affected with exactly the same symptoms and relapses.

No fever or other systemic disturbances have been observed to occur in these animals; but in the acute stages, an affected animal may suffer from such a severe pain that it may not feed so well as it normally does.

The duration of the disease varies considerably in different individuals. The acute stage lasts usually from 14 to 21 days. Relapses occur in from 1 to 6 months. Blindness ensues in these affected animals in a period varying from 6 to 18 months depending on the number and frequency of the attacks.

#### INVESTIGATIONS CONDUCTED

Cultural examination of the tears collected during the acute stages of the affection revealed a Diplococcus and a coliform organism only. These organisms and the tears when instilled on healthy eyes of experimental horses

failed to reproduce the disease. The same negative results were obtained even when these organisms were inoculated sub-conjunctivally.

The possibility of any bacteria or a virus being concerned in the causation of this condition was thus eliminated. With a view to elucidate the question of a seasonal incidence, if any, occurring in this condition which if present may be attributed to biting flies and other insects, enquiries were made and the following information was received.

*Statement showing the entries of eye-cases in Hospital since 1928*

| Year.              | Jan.      | Feb.     | Mar.      | Apr.      | May       | June      | July     | Aug.     | Sept.    | Oct.     | Nov.     | Dec.      | Total No. of cases. |
|--------------------|-----------|----------|-----------|-----------|-----------|-----------|----------|----------|----------|----------|----------|-----------|---------------------|
| 1928 . . .         | ...       | ...      | ...       | 1         | ...       | ...       | ...      | ...      | ...      | ...      | ...      | 1         | 2                   |
| 1929 . . .         | 1         | 1        | ...       | ...       | ...       | ...       | 1        | 3        | ...      | 1        | 3        | 10        |                     |
| 1930 . . .         | ...       | ...      | 1         | ...       | ...       | 1         | ...      | ...      | ...      | 3        | 3        | 3         | 11                  |
| 1931 . . .         | 2         | ...      | ...       | 2         | 5         | 2         | ...      | 1        | 3        | ...      | ...      | 1         | 16                  |
| 1932 . . .         | 2         | ...      | 1         | 1         | 2         | 1         | ...      | ...      | ...      | ...      | 4        | 5         | 16                  |
| 1933 . . .         | 4         | 1        | 7         | 6         | 4         | 4         | 2        | 2        | 2        | 3        | ...      | 4         | 23                  |
| 1934 . . .         | 5         | 2        | 1         | 2         | 5         | 2         | ...      | ...      | ...      | ...      | ...      | ...       | 17 for 6 months.    |
| <b>TOTAL . . .</b> | <b>14</b> | <b>4</b> | <b>10</b> | <b>12</b> | <b>16</b> | <b>10</b> | <b>2</b> | <b>4</b> | <b>8</b> | <b>6</b> | <b>8</b> | <b>17</b> |                     |

The above tabular statement indicates that the disease is gradually increasing. It also shows that the disease occurs at all times of the year and as such there is no evidence of a seasonal incidence attributable to it, though in the months of May, December and January, the number of cases were more numerous than during the other months.

In the colts sold to the Remount Depots from these stud farms, this eye trouble is not marked. Officers in charge of Remounts report that there is not a heavy incidence. It was observed that district mares and foals were never affected and that the incidence of this disease in Remount Depots in animals sent from these stud farms was very rare. But at Coleyana many cases occur in the young stock and the attacks terminate in blindness. The question arises as to whether the source of infection is also removed when the animals leave the Estate and whether the stock then make a complete recovery.

One is, therefore, led to consider what differences there may be in the general conditions between the Stud Farm and the Circles and it would appear to be necessary to review them briefly.

Coleyana

Circles

- |                                |   |
|--------------------------------|---|
| Concentration of stock . . .   | Dispersion of stock.  |
| Free grazing in grass paddocks | Grazing in most cases limited to crops such as lucerne etc., and grass on banks of water courses. |

*Verminous Ophthalmia in Equines*

| Coleyana  | Circles                                    |
|---|--|
| Grass in large quantities . . .   | Very limited grass.                        |
| At certain times of the year, i.e., July and August, very long grass in the paddocks which contains a large admixture of weeds. | Probably very few weeds in grazing.        |
| Mares foal in the open and young stock and mares live out in the open throughout the year.                                      | Young stock and mares stabled at night.    |
| Stock watered from tanks. . .   | Stock watered from running water channels. |

*Concentration of stock at Coleyana.*—This is particularly the case twice a day (morning and evening) when the mares are brought to the feeding troughs for inspection and grain feed. This probably leads to a concentration of flies at this time. This is especially noticeable in the afternoons in the hot weather. On the other hand, area stock are preferably grazing under trees all day and are not brought in till after dark.

*Dust.*—Very dusty practically all the year round but this was not considered to be a profitable line of enquiry.

*Horse flies.*—The mares are heavily attacked by several species of flies which settle between the mare's quarters.

History sheets of Hospital cases have been kept up since 1928 only, and it appears that the incidence of eye trouble has increased markedly since that year, but an even more marked increase has occurred between the years 1932-36.

Dietetic deficiency was suspected, but when all the details of the feeds were submitted to experts in Edinburgh they showed nothing wanting. Grasses, feeds and soils on the Estate have been repeatedly examined by Dr. Lander and the Soil Chemist at Lyallpur, but no deficiency has been brought to light.

Judging by external appearances, the condition of the average stock in the affected localities generally remains good even during the hot weather and this applies equally well to mares and to young stock. The latter, however, are subject to worm infection from about the age of 6 to 28 months, but it would appear that a resistance is set up gradually as the animals grow older.

The only two classes of animals enjoying special treatment in the Estate are the stallions and those of the stock that are taken up annually for the Delhi Horse Show. There is no recorded cases of eye disease amongst the stallions. However, the following points call for consideration. The stallions have no free grazing and they are stabled in loose boxes. The majority of the animals do not summer in the plains and they are watered with water from a well. The stallions are also carefully groomed every day and to a great extent they are protected from biting flies.





FIG. 1. Section of cornea showing a complete microfilaria in the *Substantia propria*  $\times 300$



FIG. 2. Section of cornea showing two coiled microfilaria in the *Substantia propria*. (Note the intact stratified pavement epithelium)  $\times 190$

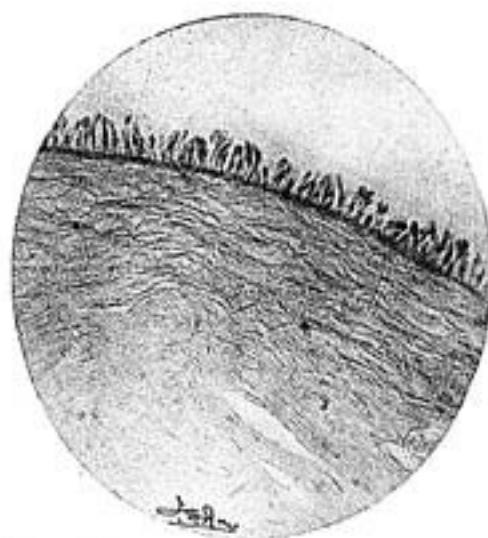


FIG. 3. Note the single layer of germinal epithelium in the cornea  $\times 190$

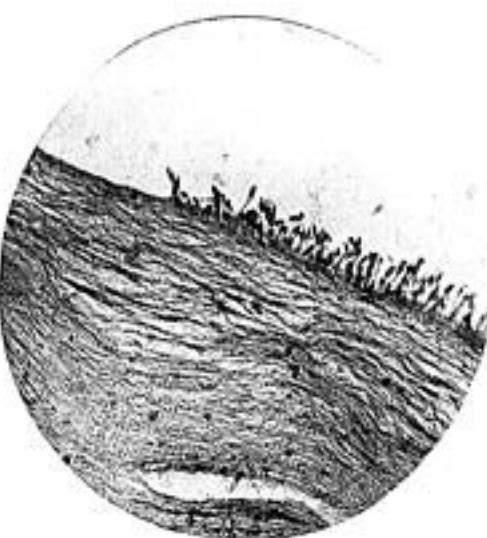


FIG. 4. Note the area completely denuded of epithelium lying continuous with the area showing a single layer  $\times 190$

In regard to the Delhi Show stock, they are brought up from the paddocks in about the month of November and are kept in loose boxes at night, but they spend most of the day tethered in the sun. They are carefully groomed and they have no free grazing. It is to be noted that no mares with foals at foot or any stock under 12 months of age are taken up for the Delhi Show.

#### ROUTINE FORM OF TREATMENT ADOPTED

1928. Boric fomentation, Boric acid and formaline in powder.
1929. Boric and zinc lotion, Boric and formaline as above.
1930. Boric and zinc sulphur, Atropine and cocaine solution.
1931. Boric and zinc sulphur, Atropine and cocaine and calomel.
1932. Boric and calomel powder, Atropine and cocaine solution and adrenaline.
1933. As above and Silver Protargol 10 grains to 1 ounce.
1934. As above and yellow oxide of mercury ointment. Mick treatment  $\frac{2}{3}$  to 5 c.c. subcutaneously every alternate day.

It may be said that none of these treatments has had any permanent result.

#### HISTOPATHOLOGICAL EXAMINATION

On the 14th May 1935, the lachrymal glands and both eyeballs of Brood mare Nili Chiri were sent to this Institute from the Probbynabad Stud Farm.

Histological examination of the lachrymal glands revealed the presence of chronic congestion, distinct eosinophilia, haemorrhagic foci, fibrotic changes in the blood vessels and oedematous changes in the parenchyma. Fairly frequent microfilariae were also seen in the stroma of the gland.

The conjunctiva and cornea showed changes associated with acute inflammation, eosinophilic, neutrophilic and lymphocytic infiltration and the presence of variable numbers of microfilariae. On finding the first microfilaria in the section of the lachrymal gland, the writer was rather doubtful as to whether these parasites were the real cause of the disease or whether their presence was more or less accidental. But their constant presence in all the following specimens, eosinophilia and fibroblastic changes demonstrated conclusively that the microfilariae were the aetiological agents.

The lachrymal glands and eyeballs of two more animals, Brood mares Nisri Jan and Amanatdarni of Probbynabad Stud Farm were also examined histologically with the same findings as those in Brood mare Nili Chiri. The preservative fluid containing the eyeballs was examined for worms and worm larvae with negative results. The lachrymal glands showed congestion, distinct endarteritis of the vessels in the supporting connective tissue, intense eosinophilia and the presence of a few sections of microfilaria. The cornea at its junction with the sclera showed distinct eosinophilia and mononuclear

infiltration and fibroblastic stimulation. The capillary blood vessels were dilated and distended with blood. A few sections of microfilaria were also seen at this junction.

The eyeballs from Nisri Jan showed evidence of verminous ophthalmia characterised by the presence of distinct eosinophilia and a few sections of microfilaria. In order that this finding may be confirmed or eliminated in cases that occur at Coleyana, it was requested that from any future case which may become available for destruction, the entire eyeballs and lachrymal glands be removed and despatched to this Institute for examination and confirmation.

The affected eyeballs from three Brood mares Viola, Kronella and Momento belonging to the Coleyana Stud Farm were also examined with the same histological features, eosinophilic infiltration and the presence of microfilaria in sections as those observed in the Probbynabad animals. The eyeballs from Brood mare Momento were sent to this Institute preserved in saline solution and this fluid yielded no microfilaria on examination; however, a few eosinophile leucocytes were seen in this fluid on examination. The corneal epithelium in this animal showed only one layer of epithelial cells in some places whereas elsewhere, the epithelium was found to be completely shed. Intense eosinophilia, mononuclear infiltration of the sclera at its junction with the cornea, and intense vascularity of the choroid coat were the other changes observed. A few microfilariae were also seen in the sections and they were situated in the *Substantia propria* of the cornea surrounded by a zone of eosinophiles and fibroblasts. Smears of eye-discharge from affected cases were examined many a time for microfilaria with negative results. Samples of tears from affected cases were submitted to chemical examination and it was found that the mineral content of the tears was abnormally high.

The finding of filarial parasites (larvae) in the materials from Probbynabad and Coleyana Farms indicates that the condition is one of verminous ophthalmia. With a view to determine the incidence of microfilariae in the eyeballs of normal horse, a few eyeballs of healthy horses were examined for microfilariae with negative results. Treatment with Antimosan and other antimony preparations was recommended in these cases. There were two fresh cases in the spring of 1936 and the symptoms shown were typical of this type of ophthalmia and they were treated quite successfully with antimosan. Neither of these two cases recurred.

#### DISCUSSION

During the recent years many different affections in horses caused by filarial larvae have been recorded in literature. *Lichen tropicus* or *khoojli* is a form of cutaneous microfilariasis affecting the horses in many parts of the world and enquiries were made as to whether any of the affected animals had ever shown lesions of *khoojli* on their skin with a view to correlate these two conditions but it was found that this latter condition has never been particularly noted in these animals.

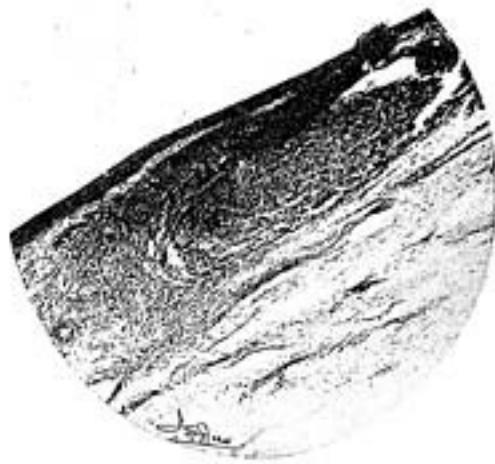


FIG. 1. Section of cornea showing intense eosinophilic infiltration surrounded by a zone of fibroblasts  $\times 60$

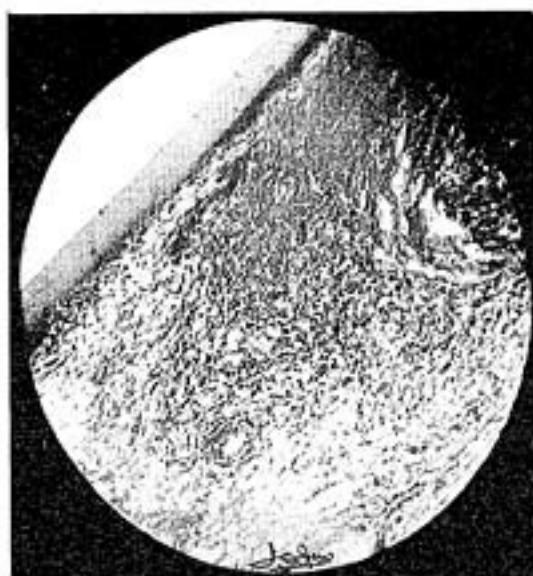


FIG. 2. Section of cornea showing intense eosinophilia surrounded by a zone of fibroblasts  $\times 140$



FIG. 3. Section of lachrymal gland showing fibrous thickening of the stroma and vessel showing marked endarteritis  $\times 40$



FIG. 4. A portion of the wall of a lachrymal duct showing intense eosinophilia  $\times 140$



None of these affected animals had ever shown worms in the eye and *Filaria oculi* are rare in the affected area.

Diseases of the eye due to filariasis are not uncommon in cattle in this country.

Venkataratnam Chetty [1922] describes a form of ulcerative keratitis which occurred in an epidemic form among the Corporation Depot Bullocks and the Buckingham Mill draught cattle in Madras in the years 1917, 1918 and 1919. The outbreak in the year 1919 resulted in loss of sight in over 40 per cent of the affected animals. The pathological changes observed varied from simple ophthalmia to ulcerative keratitis of one or both the eyes. Examination revealed a few thread like worms each about an inch long, wriggling about on the cornea and a bunch of them might be seen under the eyelid. The worms were identified as *Thelazia rhodesi*. The author suggests the possibility of their being carried from the eyes from animal to animal by flies.

Ramaswamy Iyer [1922] records a similar case of infection of the left eye of a cow with *Th. rhodesi*. The animal was lacrymating profusely for about a month and could not open the eye freely. The eye was washed with saline solution and five worms were removed. As no more worms could be found, a boric lotion bandage was applied and the animal was allowed to go. About three weeks later, the same animal was brought to his dispensary with lacrymation from both eyes. Now the eyes showed moving worms over the conjunctiva in both eyes, with slight opacity of the left cornea. Over a dozen worms were removed from both eyes. The author states that the parasites cause much irritation, severe conjunctivitis and profuse lacrymation leading on to opacity of the cornea and impairment of the vision.

Griffiths [1922] states that members of the genus *Thelazia* have their normal habitat in the lacrymal ducts of the eye of various species of mammals and birds and that some fly acts as an intermediate host and transmits the parasite to fresh mammalian hosts. The author thinks that it is among the species of *Stomoxyx*, *Musca* and allied genera that the transmitting agent may possibly be found, flies of these species being a constant source of annoyance to all domestic animals by their habits in attacking the orbital region. He adds that the presence of these parasites in the orbit gives rise to ophthalmia terminating in loss of sight.

We have not as yet been able to identify the worm responsible for the causation of this condition, and naturally its life-history is unknown. According to the Helminthologist of this Institute, "From an examination of the microfilariae and their 'unsheathed' character, it appears that the larvae belong to some Onchocercoid worm. Specific identification is not possible from the microfilariae seen in the sections".

From the data given by Steward [1935], Shafi Mohammed [1931], Cleland [1914] and Heydon, [1927] it would appear that Onchocercoid tumours need not necessarily be found in cases of Onchocercoid infections. The adult

worms may lead a free life in the tissues and deposit their embryos as they move about.

One of these cases was sent to Mukteswar from Coleyana for observation and arrived early in July 1936. This animal, Brood mare Epigram born in 1924 had four affections of the eyes, and on arrival she showed a pin-head sized cataract of the left eye and ulceration of the distal opening of the lachrymal duct of the off-nose. Apart from the cataract, her eyes appeared normal. This animal was considered a typical case of this condition by the Coleyana authorities, who thought that she would probably go blind in six months. Since arrival at Mukteswar about a year ago, this animal has not shown any signs of relapse, nor has she gone blind as was anticipated. Blood smears from this animal were examined thrice a week for some months for the presence of microfilaria, with negative results. It is possible that the cool Mukteswar climate has prevented a relapse as it is known to do in case of *Lichen tropicus* and Bursati.

Regarding diagnosis in life, Fairley [1931] describes a complement fixation reaction for filarial disease using an alcohol-soluble extract of *Dirofilaria immitis* as antigen. The intradermal reaction for *Wuchereria bancrofti* described by Taliaferro and Hoffman in 1930 was confirmed by this worker and extended further to the diagnosis of *Loa loa* and *Onchocerca volvulus* infestations. The author states that both these reactions are essentially group reactions, the scope of which probably includes all the filaroid species of the family and discusses in detail the clinical and theoretical aspects of the combined reactions.

This test is not a very reliable one for use in the diagnosis of an infection with *Onchocerca* worms, as it is not specific for this genus. The specific identity of the worm involved will be discovered only by the finding of the adult worm by means of *post mortem* examinations of affected animals.

#### SUMMARY

Under the name "Periodic Ophthalmia," a disease affecting the eyes of horses in the Stud Farms at Montgomery and Probynabad in the Punjab has been known for some years. The disease is of a recurrent nature and terminates in the affected animals going blind. The disease has no seasonal incidence as it occurs at all times of the year.

Mechanical irritation by flies and dust, bacteria and dietetic deficiency were all suspected to be concerned in the causation of this condition and every one of these individual agents was finally eliminated. Histopathological examination revealed the verminous origin of the disease and microfilariae were found in the sections of the affected eyes and lachrymal glands. The sections also revealed intense eosinophilia, neutrophile and lymphocytic infiltration and fibroblastic stimulation.

The disease is comparatively rare in the animals issued to the circles from these farms. The transmitting agent of this condition, when it is found

may possibly be one of the biting flies as there is concentration of stock in these stud farms, and this naturally leads to a concentration of flies.

From an examination of the microfilariae and their 'unsheathed' character, the larvae appear to belong to some Onchocercoid worm. Specific identification was not possible from the microfilariae seen in the sections and only *post mortem* examinations will solve the question of the identity of the adult worm, when found.

Treatment with antimosan and other antimony preparations appear to have an inhibitory effect on the progress of the disease.

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SOME EXPERIMENTS ON THE CAROTENE CONTENT OF  
GRASSES AND CONCENTRATES AND THE FEEDING  
OF GUINEA GRASS TO DAIRY COWS

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NUTRITIONAL trials have been performed to increase the vitamin and carotene content in milk and butter-fat of dairy cows. In Western countries a marked difference in carotene and vitamin A content in milk has been observed in pasture-fed as compared with stall-fed cows, and in summer and winter seasons. In India such data are not available, and except in big towns the cattle are mostly pasture fed. Vitamin A determination with milk and butter of cows of the Imperial Institute of Dairying, Bangalore, extending over one year revealed the potency to be nearly constant. However, the food and feeding conditions at the Imperial Dairy are almost ideal and the case is nearly the reverse with the farmer or the Indian *goala*. Poor as they are, they cannot afford to spare money for feeding their milch cattle except with the grasses of forests and waste lands and the remnants of by-products of crops.

The estimation of the carotene content of grasses and fodders, nevertheless, is as important as the organic or inorganic analysis of the same. In course of an analytical study of over one hundred and fifty species of grasses in Hawaii and other places, it has been observed that the analysis of a grass is of little value unless the stage of maturity at which it was cut was properly taken into account [Ripperton *et al.* 1933]. Protein is highest in immature plants and crude fibre is higher in mature ones. Watson [1935] carried out experiments on the conservation of grassland herbage and measured the losses in nutritive value during hay-making. The losses in starch and protein equivalents are serious and of about the same order. Early hay dried at a low temperature, or ensiled with acid (A. I. V. process) or molasses provided a satisfactory method of storing grassland herbage. A wide range of material containing from 10 to 20 per cent of crude protein

on the dry matter can be conserved with ease. Quick drying of hay is the least damaging as far as the contents of A, D and C vitamins are concerned [Rygh, 1934]. Silo fodder with a hydrogen ion below 4·2 is considered very good, but when the pH is above 4·9 it is considered poor [Kniest, 1934]. Exposure to sunlight and air causes a rapid decrease in the carotene content of forage [Guilbert, 1934]. A good field-cured product may approximate a dehydrated meal in "Vitamin A" value. There is little or no loss of carotene after vacuum drying for three hours at 100°C. Alternatively, by means of machine drying, it is possible to preserve grass cut at an early stage of growth and preserve the nutrients in a much larger amount than can be obtained by hay-making. They can be cut several times during the season and in this way the pigments contained in young growing stage can be retained. They contain carotene, xanthophyl and chlorophyl. The first is the precursor of vitamin A and the last two are responsible for quality. Gillam and others' experiments [1933] show that the relatively high proportion of carotene, xanthophyl and vitamin A present in summer butter can be maintained during the winter period of stall feeding by the use of artificially dried grass.

The carotene and vitamin A contents of milk and butter of cows have been found to be influenced by the breed and the diet of the animal. Carotene was highest in Guernsy cows, 10·3 γ per kg. of fat; and lowest in Holstiens, 4·3 γ. Vitamin A content varied inversely, being 5·1 in Guernsy and 10·2 in Holstien. On a low carotene diet, 3·3 per cent appeared in milk, and 1·3 per cent on a high carotene diet as vitamin A. However, individuals of the same breed differed upto 100 per cent among themselves. Increasing the carotinoid content of the diet increased, but not proportionately the carotene and vitamin A in the milk. Watson *et al* [1934] found that when 25 per cent of the concentrated foodstuff was replaced by dried grass, no improvement in the yellow colour of butter was noted. Inclusion of 50 per cent of dried grass caused distinct improvement in carotinoid colour; the inclusion of A. L. V. fodder had also the same effect.

Fraps and others [1934] have carried out experiments on the vitamin A requirement of dairy cows. They found that vitamin A recovered from the feed in the butter-fat was approximately 47 per cent in a cow receiving 8,500 units daily from yellow corn, 21 per cent in a cow getting 60,000 units for alfalfa, and 10 per cent for a cow getting 11,600 units for alfalfa and yellow corn 17,000 units daily was not enough to maintain a cow in health. One unit of vitamin A in butter required 11 units in the feed over the maintenance ration. Silage, hays and fodder will not supply enough vitamin A to maintain a high potency in butter-fat. Green growing pasture grasses are needed to maintain a high percentage of vitamin A in butter-fat.

It will be seen from the above that the estimation of carotene content of grasses and fodders is essential for a proper study of the nutrition of dairy cattle. It will then be possible to meet the carotene demand of the milch cattle with a suitable combination of feeding materials. This can also help

in judging the correct stage of maturity at which grasses are to be cut and dried or stored in silo. With the co-operation and help of the Imperial Dairy Institute and different Farm officers a number of grasses and fodders have been obtained and their carotene content determined. They represent the stage at which they are fed to the cattle. The results \* are given in Table I.

TABLE I  
*Grasses and fodders*

| No. | Name  | Place                    | Carotene<br>in mg.<br>per kg. | Remarks   |
|-----|---|--------------------------|-------------------------------|---|
| 1   | Guinea Grass I<br>( <i>Panicum maximum</i> ).                           | Bangalore<br>Dairy Farm. | 50                            | Mature green stage cut from the farm just as they are fed to the cattle.  |
| 2   | Sudan Grass [ <i>Helcys Sorghum sudanensis</i> ( <i>Piper</i> ) Hitch]. | Ditto                    | 58                            | Ditto   |
| 3   | Rhodes Grass ( <i>Chloris Gayana</i> ).                                 | Ditto                    | 30                            | Ditto   |
| 4   | Napier Grass ( <i>Pennisetum purpureum</i> ).                           | Ditto                    | 18                            | Ditto   |
| 5   | Guinea Grass II<br>( <i>Panicum maximum</i> ).                          | Ditto                    | 48                            | Ditto   |
| 6   | Lucerne ( <i>Medicago sativa</i> ).                                     | Ditto                    | 168                           | Ditto   |
| 7   | Jowar [ <i>Aandropogon Sorghum</i> ].                                   | Ditto                    | 40                            | Ripe mature stage when they are cut for the silo or daily feed.   |
| 8   | Paspalum ( <i>Paspalum dilatatum</i> ) Italian Blue Grass.              | Ditto                    | 200                           | This grass is very much liked by the dairy cattle. The colour of the butter from this feed is also deep yellow in tint. |
| 9   | Mauritius ( <i>Panicum maximum</i> ).                                   | Ditto                    | 100                           | Ditto   |
| 10  | II U. B.  | Ditto                    | 100                           | Ditto   |

\*The feeding quality of the fodders depends to a great extent on the stage of maturity at which the crop is cut. As this information is not available in some cases one will have to be careful before using the carotene data, as given by authors, for calculating the Vitamin A potency of these samples.—Ed.

*Carotene Content of Grasses*

TABLE I—*contd.*  
*Grasses and fodders*

| No. | Name  | Place   | Carotene<br>in mg.<br>per kg. | Remarks   |
|-----|---|---|-------------------------------|---|
| 11  | Silo ( <i>Panicum maximum</i> ).                                    | Bangalore<br>Dairy Farm.                            | 4                             | Sample taken from the daily feed.   |
| 12  | Ragi ( <i>Eleusine coracana</i> ).                                  | Cattle Breed-<br>ing Farm,<br>Bankapur,<br>Dharwar. | 96                            | Flowering stage. Two months,<br>3 weeks old. Height when cut<br>2 to 1 ft.  |
| 13  | Bajri (local) ( <i>Pennisetum typhoideum</i> ).                     | Ditto   | 48                            | Grains were forming. Two months 3 weeks duration. Height when cut 4 to 8 ft.  |
| 14  | Bajri (African variety) ( <i>Pennisetum typhoideum</i> ).           | Ditto   | 42                            | Height when cut 3 to 7 ft. Grains were forming 2 months 3 weeks old.  |
| 15  | Maize (American) ( <i>Zea Mays</i> ).                               | Ditto   | 40                            | Ripening stage. Height when cut 3 to 5 ft. Two months 3 weeks old.  |
| 16  | Kulthi (Horse Gram) ( <i>Dolichos biflorus</i> ).                   | Ditto   | 140                           | Flowering stage. Two months old. Height 1 to 1½ ft.   |
| 17  | <i>Rouya Incana</i> .   | Ditto   | 54                            | Grains developing. Three months 3 weeks old. Height 3 to 8 ft.  |
| 18  | <i>Shevari</i> .  | Ditto   | 158                           | Height when cut for sample was 2 ft. and is the second cutting one month after the first cutting done 3 months after sowing.          |
| 19  | Elephant Grass ( <i>Pennisetum purpureum</i> ).                     | Ditto   | 70                            | Height when cut for sampling was 6 in. to 1 ft. 6 in. Planted 5 months before and cut 4 times.  |
| 20  | Gordura Grass (Brazilian variety) ( <i>Melinis minuti- flora</i> ). | Ditto   | 88                            | Plants were 4 months old, and the samples are from the second cutting: 1st cutting was done 3 months after sowing. Height 1 ft. only. |
| 21  | Garag grass (Brazilian variety).                                    | Ditto   | 120                           | Plants were 4 months old, and samples were from the 2nd cutting, one month after the 1st cutting. Height 2 ft. only.                  |

TABLE I—contd.

## Grasses and fodders

| No. | Name   | Place                     | Carotene<br>in mg.<br>per kg. | Remarks  |
|-----|--|---------------------------|-------------------------------|--|
| 22  | Methi ( <i>Trigonella foenum-graecum</i> ).          | Govt. Farm,<br>Ranchi.    | 58                            | Height 1 ft. 6 in. with flowers<br>and pods.                         |
| 23  | Elephant Grass<br>( <i>Pennisetum purpureum</i> ).   | Ditto .                   | 88                            | Cut at early stage at 4 in.<br>height.                               |
| 24  | Soya bean (green<br>fodder) ( <i>Glycine Soja</i> ). | Ditto .                   | 114                           | Height 1 ft. with flowers and<br>pods.                               |
| 25  | Maize (green fodder)<br>( <i>Zea Mays</i> ).         | Ditto .                   | 48                            | Height 5 ft. 3 in. with flowers.                                     |
| 26  | Cow Pea ( <i>Vigna Catjang</i> ).                    | Ditto .                   | 182                           | Height 9 in. before flowering.                                       |
| 27  | Peas ( <i>Pisum sativum</i> )                        | Ditto .                   | 80                            | Height 1 ft. 6 in. with flowers<br>and pods.                         |
| 28  | Elephant grass<br>( <i>Pennisetum purpureum</i> ).   | Govt. Farm,<br>Sabour.    | 100                           | Height 2 to 2½ ft. Two months<br>after last cutting.                 |
| 29  | Berseem ( <i>Trifolium alexandrinum</i> ).           | Ditto .                   | 90                            | One month old. First cutting<br>at 1 to 1½ ft. height.               |
| 30  | Kalai ( <i>Phaseolus radiatus</i> ).                 | Ditto .                   | 46                            | Three months old. Approaching<br>maturity. Height 9 in. to<br>11 in. |
| 31  | Rice straw ( <i>Oryza sativa</i> ).                  | Ratnagiri<br>Farm.        | Nil                           |  |
| 32  | Nagales straw ( <i>Eleusine coracana</i> ).          | Ditto .                   | Nil                           |  |
| 33  | Rannatki ( <i>Phaseolus trilobus</i> ).              | Govt. Farm,<br>Kopergaon. | 88                            | Green.   |
| 34  | Shewari . . .  | Dharwar .                 | 40                            |  |
| 35  | Water Hyacinth<br>( <i>Eichhornia crassipes</i> ).   | Dacca L. S. F.            | 58                            |  |

D

*Carotene Content of Grasses*

TABLE I—contd.

*Grasses and fodders*

| No. | Name  | Place                                 | Carotene<br>in mg.<br>per kg. | Remarks  |
|-----|---|---------------------------------------|-------------------------------|--|
| 36  | Fresh hay . . .                                 | Agric. Station,<br>Surat.             | 10                            | Representative sample from a mixture of hay fed to dairy cattle. The grasses were in ripe seed stage.                      |
| 37  | Maize fodder ( <i>Zea Mays</i> ).               | Arbhari Farm                          | Trace                         |  |
| 38  | Rice straw ( <i>Oryza sativa</i> ).             | Ditto . .                             | Ditto                         |  |
| 39  | Shewari . . .                                   | Ditto . .                             | 140                           |  |
| 40  | Elephant Grass ( <i>Pennisetum purpureum</i> ). | Cuttack, Dir. of Agriculture, Orissa. | 36                            | 54 in. height. Growing stage. Arrived infected with moulds.  |
| 41  | Biri plants . . .                               | Ditto . .                             | 130                           | 17 in. height. Flowering stage. Arrived infected with moulds.  |
| 42  | Cow pea plants ( <i>Vigna Catjang</i> ).        | Ditto . .                             | 140                           | 19 in. height. Flowering stage. Arrived infected with moulds.  |
| 43  | <i>Dolichos Lablab</i> . .                      | Millets Specialist, Coimbatore.       | 130                           | The plants were in flowering stage. They were cut at ground level. Cut up in pieces. Arrived in slightly mouldy condition. |
| 44  | <i>Dolichos biflorus</i> . .                    | Ditto . .                             | 120                           | Ditto  |
| 45  | <i>Sorghum versicolor</i> .                     | Ditto . .                             | 76                            | Ditto  |
| 46  | <i>Sorghum purpureo-serratum</i> .              | Ditto . .                             | 90                            | Ditto  |
| 47  | <i>Pennisetum typhoides</i> .                   | Ditto . .                             | 56                            | Ditto  |
| 48  | <i>Sorghum halepense</i>                        | Ditto . .                             | 32                            | Ditto  |
| 49  | <i>Setaria italica</i> . .                      | Ditto . .                             | 120                           | Ditto  |
| 50  | <i>Panicum miliare</i> . .                      | Ditto . .                             | 104                           | Ditto  |
| 51  | <i>Sesbania grandiflora</i> .                   | Ditto . .                             | 202                           | Ditto  |

TABLE I—*contd.*  
*Grasses and fodders*

| No. | Name   | Place                             | Carotene<br>in mg.<br>per kg. | Remarks   |
|-----|--|-----------------------------------|-------------------------------|---|
| 52  | <i>Vigna Catjang</i>   | Millets Specialist, Coimbatore.   | 114                           | The plants were flowering stage. They were cut at ground level. Cut up in pieces. Arrived in slightly mouldy condition. |
| 53  | <i>Ipomea hispida</i>  | Ditto                             | 88                            | Ditto   |
| 54  | Maize (Golden beauty) ( <i>Zea Mays</i> )                      | Agricultural College, Coimbatore. | 24                            | Flowering stage. Height 5 ft. 6 in. Loss of moisture on drying 80 per cent.   |
| 55  | Teosinte   | Ditto                             | 38                            | Flowering stage. Cut 2 in. above ground. Height of plant 4 ft. 6 in. Loss on drying 80 per cent.                        |
| 56  | Elephant Grass ( <i>Pennisetum purpureum</i> ).                | Ditto                             | 28                            | Full grown and nearly flowering. Height of plant 6 ft. Cut in 2 in. above ground. Loss on drying 80 per cent.           |
| 57  | Ordinary grass from forest area.                               | Kumta Farm                        | Nil                           | Mature, 2½ ft. high. Dry grazing feed from October to April.  |
| 58  | Rice straw ( <i>Oryza sativa</i> ).                            | Ditto                             | Nil                           | After removal of paddy. Dry feed from May to October. Three ft. high.   |
| 59  | Karki. Grown in salt land creeks. ( <i>Celtis caucasica</i> ). | Ditto                             | 46                            | Height 20 in. Green weeds from November to June.  |
| 60  | Udid chaff ( <i>Phaseolus radiatus</i> ).                      | Ditto                             | Nil                           | After removal of adid grains the chaff is fed in rainy season.  |
| 61  | Darodi hay   | Dohad Farm                        | 16                            | Ripe dry stage.   |
| 62  | Bathi hay  | Ditto                             | 30                            | Ditto   |
| 63  | Moshi hay  | Ditto                             | 36                            | Ditto   |
| 64  | Jinjro hay   | Ditto                             | 20                            | Ditto   |

*Carotene Content of Grasses*TABLE I—*concl'd.**Grasses and fodders*

| No. | Name  | Place                    | Carotene<br>in mg.<br>per kg. | Remarks   |
|-----|---|--------------------------|-------------------------------|---|
| 65  | Spear Grass<br>( <i>Heteropogon con-</i><br><i>tortus</i> ).<br>hay         | Hosur Cattle Farm.       | 14                            |   |
| 66  | Spear Grass green<br>( <i>Heteropogon con-</i><br><i>tortus</i> ).<br>green | Ditto                    | 34                            |   |
| 67  | <i>Andropogon annula-</i><br><i>tus</i> (green).                            | Ditto                    | 44                            |   |
| 68  | Kolukkatti Grass<br>( <i>Andropogon annula-</i><br><i>tus</i> ) (green).    | Ditto                    | 118                           |   |
| 69  | <i>Chloris barbata</i><br>(green).  | Ditto                    | 40                            |   |
| 70  | <i>Indigofera anna-</i><br><i>phylla</i> (green).                           | Ditto                    | 52                            |   |
| 71  | Rice straw ( <i>Oryza sativa</i> ).   | Nagpur Agric. Depot.     | Trace                         | Height 4 in. Harvested before dead ripe.            |
| 72  | Groundnut stems and leaves<br>( <i>Arachis hypogaea</i> ).                  | Ditto                    | 10                            | Height 2½ ft. Harvested in green condition.         |
| 73  | Berseem ( <i>Trifolium alexandrinum</i> ).                                  | Govt. Agric. Farm, Patna | 78                            | Height 1 ft. 9 in. Cut after 34 days from planting. |

A number of concentrate feeds were also analysed and the results are shown in Table II.

TABLE II

## Concentrates

| No. | Name   | Place                         | Carotene<br>in mg.<br>per kg. |
|-----|--|-------------------------------|-------------------------------|
| 1   | Maize . . . . .                                | Central Poultry Farm, Kirkee. | Nil                           |
| 2   | Mammoth Yellow Soya Bean . .                   | Ditto                         | Trace                         |
| 3   | Fish meal . . . .                              | Ditto                         | Nil                           |
| 4   | African Bajri . . . .                          | Ditto                         | Trace                         |
| 5   | Brewer's grain . . . .                         | Bangalore Dairy Farm          | Nil                           |
| 6   | Mustard cake . . . .                           | L. S. E., Dacca . .           | Nil                           |
| 7   | Til cake . . . .                               | Ditto                         | Nil                           |
| 8   | Maize . . . . .                                | Govt. Farm, Ranchi . .        | Nil                           |
| 9   | Mustard cake . . . .                           | Govt. Farm, Sabour . .        | Nil                           |
| 10  | Mustard cake . . . .                           | Govt. Farm, Cuttack . .       | Trace                         |
| 11  | Mustard cake . . . .                           | Govt. Cattle Farm, Gauhati.   | Trace                         |
| 12  | Mati Kola flour . . . .                        | Ditto                         | Nil                           |
| 13  | Tur chunni ( <i>Cajanus indicus</i> ) .        | Surat Farm . . . .            | Nil                           |
| 14  | Guar (beans) ( <i>Cyamopsis psoroides</i> ). . | Ditto . . . .                 | Nil                           |
| 15  | Til oil-cake . . . .                           | Ditto . . . .                 | Nil                           |
| 16  | Cotton seed . . . .                            | Ditto . . . .                 | Nil                           |
| 17  | Maize seed . . . .                             | Arbhari Farm . . . .          | Nil                           |
| 18  | Kulthi seed . . . .                            | Ditto . . . .                 | Nil                           |
| 19  | Cotton seed . . . .                            | Govt. Farm, Kopergaon . .     | Nil                           |
| 20  | Matki . . . . .                                | Ditto                         | Nil                           |
| 21  | Hulga . . . . .                                | Ditto                         | Nil                           |

TABLE II—*contd.**Concentrates*

| No. | Name                  | Place                           | Carotene<br>in mg.<br>per kg. |
|-----|-----------------------|---------------------------------|-------------------------------|
| 22  | Safflower cake        | Govt. Farm, Kopergaon.          | Nil                           |
| 23  | <i>Udid Vato</i>      | Dohad Farm                      | Trace                         |
| 24  | Til cake              | Ditto                           | Nil                           |
| 25  | Kurasihi cake         | Kumta Farm                      | Trace                         |
| 26  | Coconut cake          | Ditto                           | Nil                           |
| 27  | Gowar seed            | Ditto                           | Nil                           |
| 28  | Cotton seed           | Ditto                           | Nil                           |
| 29  | Soya bean             | Hosur Cattle Farm               | Trace                         |
| 30  | Ragi grain            | Coimbatore Cattle Farm          | Nil                           |
| 31  | Linseed oil-cake      | Nagpur Cattle Farm              | Nil                           |
| 32  | <i>Tur chunni I</i>   | Ditto                           | Nil                           |
| 33  | <i>Tur chunni II</i>  | Ditto                           | Nil                           |
| 34  | <i>Tur chunni III</i> | Ditto                           | Trace                         |
| 35  | Masur (Lentil)        | Ditto                           | Nil                           |
| 36  | Black til oil-cake    | Ditto                           | Nil                           |
| 37  | White til oil-cake    | Ditto                           | Nil                           |
| 38  | Juar meal             | Ditto                           | Trace                         |
| 39  | Lakh Tiwara           | Ditto                           | Nil                           |
| 40  | Barley                | Imperial Dairy Farm, Bangalore. | Nil                           |

TABLE II—*concl.**Concentrates*

| No. | Name                       | Place                           | Carotene<br>in mg.<br>per kg. |
|-----|----------------------------|---------------------------------|-------------------------------|
| 41  | Gram . . . . .             | Imperial Dairy Farm, Bangalore. | Nil                           |
| 42  | Horse Gram . . . . .       | Ditto                           | Nil                           |
| 43  | Linseed . . . . .          | Ditto                           | Nil                           |
| 44  | Rice bran . . . . .        | Ditto                           | Nil                           |
| 45  | Karai . . . . .            | Ditto                           | Nil                           |
| 46  | Wheat bran . . . . .       | Ditto                           | Nil                           |
| 47  | Oats . . . . .             | Ditto                           | Trace                         |
| 48  | Cotton seed-meal . . . . . | Ditto                           | Nil                           |
| 49  | Wheat . . . . .            | Ditto                           | Trace                         |
| 50  | Gram chunni . . . . .      | Ditto                           | Nil                           |
| 51  | Thoor husk . . . . .       | Ditto                           | Nil                           |
| 52  | Groundnut cake . . . . .   | Ditto                           | Nil                           |

To find out the effect of carotinoid feed on dairy cows, three groups of two cows each were given basal ration and guinea grass. The first group was given the normal ration, the second group was given twenty per cent extra guinea grass and the straw supply was omitted. The third group was provided guinea grass fourteen per cent less than normal and the deficit was made up with straw. The milk-yield, carotene, and vitamin-content of butter and the carotene-content of the blood of the animals are given for weekly samples. The experiments were carried on for five weeks and the results are given in the Tables III and IV.

TABLE III  
Statement of consumption of guinea grass by the experimental cows and milk-yield and fat percentage

| Week ending         | Group A         |        |         | Group B                   |        |         | Group C         |        |         |
|---------------------|-----------------|--------|---------|---------------------------|--------|---------|-----------------|--------|---------|
|                     | Cow Nos.<br>256 | 257    | Average | Cow Nos.<br>247           | 263    | Average | Cow Nos.<br>274 | 276    | Average |
| 10th September 1936 | 31.7            | 30.85  | 31.275  | 28.07                     | 41.0   | 34.955  | 24.42           | 21.14  | 22.78   |
| 17th September 1936 | 34.14           | 32.07  | 33.115  | 38.42                     | 40.5   | 39.46   | 26.4            | 23.25  | 24.80   |
| 24th September 1936 | 34.17           | 21.42  | 32.795  | 41.28                     | 41.0   | 41.14   | 27.6            | 28.71  | 28.1    |
| 1st October 1936    | 35.0            | 35.0   | 35.0    | 41.92                     | 42.5   | 42.21   | 29.5            | 29.57  | 29.55   |
| 8th October 1936    | 34.35           | 34.71  | 34.58   | 41.73                     | 42.5   | 42.14   | 27.71           | 30.0   | 28.855  |
| Average             | 34.11           | 33.29  | 33.20   | 39.89                     | 41.11  | 39.89   | 27.71           | 28.852 | 28.852  |
|                     |                 |        |         | Milk-yield per cow in lb. |        |         |                 |        |         |
| 11th September 1936 | 10.57           | 12.457 | 11.468  | 21.657                    | 21.357 | 21.407  | 17.402          | 20.357 | 18.90   |
| 18th September 1936 | 10.429          | 13.07  | 11.749  | 23.428                    | 21.928 | 22.178  | 16.142          | 20.7   | 18.421  |
| 25th September 1936 | 10.57           | 12.367 | 11.468  | 23.57                     | 21.785 | 22.177  | 15.97           | 21.57  | 18.570  |
| 2nd October 1936    | 10.9            | 12.07  | 11.93   | 22.367                    | 21.785 | 22.071  | 15.285          | 21.765 | 18.655  |
| 9th October 1936    | 10.214          | 12.142 | 11.17   | 21.785                    | 21.357 | 21.571  | 14.5            | 21.57  | 18.053  |
| Average             | 10.55           | 11.437 | 11.437  | 21.92                     | 21.92  | 21.92   | 16.512          | 21.92  | 18.512  |
|                     |                 |        |         | Fat Percentage            |        |         |                 |        |         |
| 24th September 1936 | 5.2             | 5.3    | 5.35    | 4.65                      | 6.2    | 5.42    | 4.0             | 5.35   | 5.125   |
| 10th September 1936 | 6.35            | 6.3    | 6.375   | 4.65                      | 5.15   | 4.9     | 4.25            | 4.45   | 4.35    |
| 22nd September 1936 | 5.16            | 5.25   | 5.2     | 4.5                       | 4.7    | 4.6     | 4.4             | 4.5    | 4.45    |
| 30th September 1936 | 5.15            | 5.25   | 5.2     | 4.65                      | 4.65   | 4.6     | 4.45            | 4.65   | 4.55    |
| 7th October 1936    | 5.06            | 5.10   | 5.025   | 4.7                       | 5.3    | 5.0     | 4.3             | 4.45   | 4.375   |
| Average             | 5.11            | 5.27   | 5.27    | 4.81                      | 5.11   | 5.04    | 4.47            | 4.57   | 4.57    |
|                     |                 |        |         | Weight of animals in lb.  |        |         |                 |        |         |
| 22nd September 1936 | 720             | 601    | 673     | 675                       | 641    | 601     |                 |        |         |
| 2nd October 1936    | 734             | 654    | 682     | 660                       | 652    | 600     |                 |        |         |
| 6th October 1936    | 737             | 650    | 695     | 663                       | 651    | 592     |                 |        |         |

TABLE IV

*Carotene content in blood in mg. per c.c.*

| Date            | Group A  |          | Group B  |         | Group C  |         |
|-----------------|----------|----------|----------|---------|----------|---------|
|                 | Cow Nos. |          | Cow Nos. |         | Cow Nos. |         |
|                 | 256      | 257      | 247      | 263     | 274      | 278     |
| 8th Sept. 1936  | 0.00027  | 0.000245 | ..       | ..      | 0.00032  | 0.00046 |
| 16th Sept. 1936 | 0.0004   | 0.00041  | 0.00035  | 0.00048 | ..       | 0.00048 |
| 22nd Sept. 1936 | 0.00055  | 0.00076  | ..       | ..      | ..       | 0.00048 |
| 28th Sept. 1936 | 0.00088  | 0.00091  | 0.00068  | 0.00053 | 0.000565 | 0.00004 |
| 1st Oct. 1936   | 0.0022   | 0.00180  | 0.00117  | 0.00124 | 0.00126  | 0.00103 |
| 6th Oct. 1936   | 0.00216  | 0.00184  | 0.00109  | 0.00121 | 0.00114  | ..      |

*Carotene in ghee*

|                 |        |        |        |
|-----------------|--------|--------|--------|
| 5th Sept. 1936  | 0.0039 | 0.0044 | 0.0039 |
| 11th Sept. 1936 | 0.0021 | 0.0048 | 0.0046 |
| 20th Sept. 1936 | 0.0028 | 0.0051 | 0.0058 |
| 25th Sept. 1936 | 0.0044 | 0.0060 | 0.0042 |
| 30th Sept. 1936 | 0.0056 | 0.0060 | 0.0052 |
| 5th Oct. 1936   | 0.0064 | 0.0064 | 0.0056 |

*Vitamin A content in ghee in Lovibond Blue Units per gram*

|                 |      |      |      |
|-----------------|------|------|------|
| 5th Sept. 1936  | 14.4 | 15.4 | 14.3 |
| 11th Sept. 1936 | 13.3 | 14.4 | 14.3 |
| 20th Sept. 1936 | 12.3 | 15.4 | ..   |
| 25th Sept. 1936 | ..   | 14.3 | 13.3 |
| 30th Sept. 1936 | 14.3 | ..   | 14.4 |
| 5th Oct. 1936   | 14.4 | 15.5 | 14.4 |

## METHODS

*Estimation of carotene.*—Carotene has been estimated colorimetrically or spectrophotometrically in solution after extraction. Barnett [1934] and Gillam [1934] have used spectrophotometric method for the assay of carotene in butter. De [1935] uses the same method with vegetables. Guillet [1934] and Ferguson and Bishop [1934] have used the colorimetric method for the estimation of carotene in vegetable products. The last method has been followed in our experiments. For the assay of carotene in grasses and fodders, the material was finely chopped, dried and then powdered. Two to ten grams of the material depending on the richness of the carotene content were digested for one hour under reflux in twenty per cent alcoholic potash. The flask was then cooled and the contents filtered. The residue was washed

with ether-saturated water till the washings were free from colour. The residues were again extracted with acetone till the extracts were free from colour. The aqueous and acetone extracts were then extracted with petroleum ether, washed with water, dehydrated and made up to a volume. A colorimetric reading was then taken of the total carotinoid content in a Lovibond Tintometer, or against a standard bichromate solution. The carotene content was calculated from Ferguson's curves. The carotinoids consist of carotene and xanthophyl and the proportions of the two were determined by the 'methyl alcohol petroleum ether' partition method. In another rapid method by Pyke [1936] the two processes are combined into one and the extraction is performed in a mixture of ether and methyl alcoholic potash solution. The fraction in ether contains the carotene. One hundred mg. of grass-meal was finely ground and shaken vigorously for five minutes in a centrifuge tube with 10 c.c. of ether and 3 c.c. of 25 per cent solution of potassium hydroxide in methyl alcohol. The ether-layer was drawn off, washed with water and filtered in a sintered glass funnel over anhydrous sodium sulphate. The process was repeated till there was no more of extraction possible either from the residue or the methyl-alcohol-layer. The extract was evaporated and the residue dissolved out again in 25 c.c. of ether and an equal volume of 85 per cent methyl-alcohol. The upper ether-layer contained the carotene and the lower alcoholic layer xanthophyl. One or both the methods were tried in the case of grasses and fodders and with the necessary modification in the case of other feeding materials.

In the case of blood, Palmer's method has been followed. About 5 c.c. of blood was drawn from the animal and centrifuged to separate the corpuscles. 2 c.c. of serum was then mixed with about 8 grm. of Plaster of Paris. The paste was then extracted with a mixture of 10 c.c. of alcohol and 5 c.c. of petroleum ether. The extraction was repeated with ether till no more coloured extracts were obtainable. The ether-extract was then washed, dehydrated and colour strength matched against a standard bichromate solution to obtain the carotene content.

The carotene content was determined in butter after saponification, on the unsaponifiable matter.

Six Sindhi young cows of almost equal age and second lactation were selected for the feeding trials. The following basic ration was given per cow, each day—

|   |        |
|---|--------|
| Guinea grass . . . . .  | 35 lb. |
| Ragi straw . . . . .  | 4½ lb. |
| Groundnut-cake . . . . .  | 12 oz. |
| Concentrate mixture one pound for two pounds of milk-yield per day. |        |

Group A was given the above ration. Group B was fed with 46½ lb. of guinea grass and the straw was excluded. Group C was provided with 30 lb. of guinea grass and 6½ lb. of straw. The actual quantity of guinea

grass consumed was obtained by subtracting the residue from the quantity provided. The milk-yield was measured each day and the fat percentage was calculated on weekly averages. A weekly average sample of butter was used for carotene and vitamin A estimation. The blood was drawn once a week for carotene estimation.

#### DISCUSSION AND SUMMARY

Karrer and Schlientz [1934] have shown that carotene from grasses, spinach and nettles may be regarded as pure  $\beta$  carotene. Smith and Milner [1934] found leaf (alfalfa) carotene to consist mostly of  $\beta$  carotene. Again  $\beta$  carotene produces vitamin A in the physiological system in equivalent amount and  $\alpha$  or  $\gamma$  carotene produces vitamin A in only half the amount. Apparently, therefore, the variation in the form of carotene occurring in common forage plants is a negligible factor in expressing vitamin A value from the carotene content.

In the process of drying for analysis with hot air at 45°C. for 24 hours the carotene loss was very little. Some of the samples that were sent in the green stage sometimes became a little mouldy and suffered a little loss on this account. Duplicate trials have proved that the method followed yielded reliable and concordant results.

In a feeding trial for a short period, it is not possible to deduce any definite conclusions. In a trial on lactating cows it is not possible to measure the vitamin A reserve before or after the experiment. The stored up vitamin A is so slowly used up when carotene supply is in deficit, that no detectable difference can be noted in milk [Schieblisch, 1932]. However, the yellow value in butter and the carotene content of blood go to show that increased carotinoid feeding helps to build up reserve of vitamin A in the animal and a slight increase in the milk supply. Further intensive work is necessary to solve the many complicated phases of the problem. It would, of course, be desirable to know which breed of cattle in India produces milk richest in vitamin content.

We wish to take this opportunity to thank the Directors of Agriculture of various provinces in India and other gentlemen, too numerous to be individually mentioned here, who very kindly supplied us samples of grasses, fodders and concentrates. Thanks are also due to Mr. Zal R. Kothavalla, the Imperial Dairy Expert, for this valuable suggestions and criticisms and to Dr. V. Subrahmanyam, Professor of Biochemistry, Indian Institute of Science, Bangalore, but for whose co-operation, this work could not have been undertaken. Dr. G. S. Siddappa very kindly performed a number of determinations and thus helped us in checking and duplicating many of the carotene values in this paper to test the accuracy of the methods employed.

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# A COMPARATIVE STUDY OF THE COLOSTRUM OF THE DAIRY COW AND THE DAIRY BUFFALO

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No study has so far been made of the composition of the colostrum of buffaloes, and the analysis of the colostrum of the Indian dairy cow has been made only in the Montgomery breed, by Shah [1936]. In this experiment a more detailed analysis of the colostrum of cows and buffaloes, than has hitherto been done by any previous worker, has been carried out with a view to make a comparative study of the colostrum of these two types of dairy animals.

The most striking difference between normal milk and colostrum is in the amount of globulin, a protein that occurs in blood and in traces only in ordinary milk, but is present in colostrum in a comparatively large amount. This globulin content has been found to correspond perfectly with the amount of hemolysins in colostrum [Rogers, 1935] and other workers [Mason, Dalling and Gordon, 1930 and Davies, 1936] have shown that the transmission of antibodies from the mother to the offspring is through the globulin of colostrum and that there is no transmission of maternal antibodies through the placenta.

The animals selected for the investigation were from the two important Indian dairy breeds maintained at the Imperial Dairy Institute Farm, Bangalore, viz., three Sindhi cows and three Murrab buffaloes. Samples of colostrum were taken immediately after calving, and every six hours for the first day after calving, every twelve hours for the next two days, and after twenty-four hours on the fourth day, by which time the milk was found to have become normal. Samples were drawn from all the four quarters of the udder.

## ANALYTICAL METHODS

The determination of the proteins was carried out by the newer methods of protein analysis suggested by Moir, and adopted by Bergman and Turner [1937] in their analysis of goats' colostrum.

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The total protein was precipitated by warm trichloracetic acid, thereby leaving the non-protein nitrogen in solution. After filtering and washing several times with a dilute solution of trichloracetic acid, the filter paper and precipitate were transferred to a Kjeldahl flask, and the nitrogen content was determined by the usual Kjeldahl method.

Casein was precipitated by dilute acetic acid buffered with a solution of sodium acetate to maintain the pH at approximately 4·6, which is the pH of casein. The precipitate was filtered and washed with distilled water, and the filter paper with the precipitate was digested in a Kjeldahl flask to estimate the nitrogen content as before.

Casein and globulin were precipitated together by the addition of a saturated solution of magnesium sulphate, after first neutralising the sample to phenolphthalein with sodium hydroxide. An additional amount of anhydrous magnesium sulphate was added to saturate the water in the sample. After the precipitation was complete, the solution was filtered in a pressure filter and the residue was washed with a saturated salt solution. The nitrogen content was determined as for total protein.

The amount of albumin was calculated by subtracting casein and globulin from total protein, while the difference between casein and globulin and casein gave the globulin content of the sample.

The specific gravity was determined by the Westphal balance at a temperature of 20°C.; acidity and chloride by titrating with sodium hydroxide and silver nitrate respectively; fat by the Gerber method; total solids and ash respectively by evaporating to complete dryness on a water bath, and subsequent ashing of the residue; and lactose by titrating with the sample, a 6 per cent copper sulphate solution saturated with a mixture of potassium thiocyanate, sodium carbonate and sodium phosphate in the ratio of 3 : 6 : 10; 0·4 gm. of lactose reducing 5 c.c. of the solution.

TABLE I  
*Composition of the colostrum of Murrah buffaloes*

|  | 0<br>(At<br>birth) | 0     | No. of hours after parturition |       |       |       |       |       |       |  |
|--|--------------------|-------|--------------------------------|-------|-------|-------|-------|-------|-------|--|
|  |                    |       | 0<br>(amp.<br>of 12 &<br>18)   | 24    | 36    | 48    | 60    | 72    | 96    |  |
| Specific gravity   | 1·074              | 1·027 | 1·065                          | 1·040 | 1·046 | 1·042 | 1·038 | 1·036 | 1·035 |  |
| Acidity (c.e.s. of N <sub>2</sub> alkali required to neutralise 10 c.c.) | 0·35               | 0·43  | 0·43                           | 0·33  | 0·20  | 0·27  | 0·21  | 0·19  | 0·17  |  |
| Percentages  |                    |       |                                |       |       |       |       |       |       |  |
| Chloride (as NaCl)   | 40·6               | 38·8  | 30·0                           | 30·51 | 30·46 | 30·51 | 30·36 | 30·21 | 30·22 |  |
| Water  | 21·1               | 68·0  | 68·6                           | 78·5  | 81·4  | 80·4  | 83·3  | 82·8  | 84·0  |  |
| Total solids   | 28·9               | 31·0  | 31·4                           | 21·5  | 18·6  | 19·6  | 18·6  | 17·2  | 16·0  |  |
| Fat  | 3·9                | 4·0   | 6·2                            | 7·5   | 7·6   | 9·3   | 7·1   | 7·8   | 7·0   |  |
| Total protein (N × 6·38)   | 21·4               | 22·8  | 18·4                           | 10·9  | 7·4   | 6·6   | 5·3   | 5·1   | 4·4   |  |
| Casein (N × 6·38)  | 6·8                | 7·7   | 6·1                            | 5·5   | 4·2   | 4·2   | 3·9   | 4·2   | 4·0   |  |
| Albumin  | 3·8                | 3·6   | 3·3                            | 3·0   | 2·7   | 2·6   | 2·6   | 2·2   | 2·3   |  |
| Globulin   | 10·8               | 12·5  | 9·0                            | 2·7   | 1·0   | 0·8   | 0·2   | 0·1   | 0·1   |  |
| Lactose  | 2·2                | 2·2   | 2·4                            | 2·5   | 2·7   | 3·0   | 3·2   | 3·4   | 3·7   |  |
| Ash  | 1·1                | 0·9   | 0·8                            | 0·7   | 0·8   | 0·7   | 0·7   | 0·7   | 0·7   |  |
| Coag. on boiling   | +                  | +     | +                              | +     | +     | —     | —     | —     | —     |  |
| Coag. with rennet  | —                  | —     | —                              | —     | —     | +     | +     | +     | +     |  |

TABLE II

*Composition of the colostrum of Sindhi cows*

|  | 0<br>(gal.<br>litre) | No. of hours after parturition |                        |       |       |       |       |       |       |
|--|----------------------|--------------------------------|------------------------|-------|-------|-------|-------|-------|-------|
|  |                      | 6                              | Comp.<br>of 12 &<br>18 | 24    | 36    | 48    | 60    | 72    | 96    |
| Specific gravity   | 1.063                | 1.053                          | 1.043                  | 1.038 | 1.037 | 1.035 | 1.033 | 1.035 | 1.031 |
| Acidity (per cent. of N, alkali required to neutralise 10 per cent.) | 0.41                 | 0.35                           | 0.32                   | 0.32  | 0.29  | 0.25  | 0.23  | 0.22  | 0.20  |
| Percentages  |                      |                                |                        |       |       |       |       |       |       |
| Chloride (as NaCl)   | 0.46                 | 0.44                           | 0.36                   | 0.33  | 0.35  | 0.36  | 0.33  | 0.31  | 0.29  |
| Water  | 81.8                 | 81.1                           | 82.2                   | 81.4  | 85.3  | 87.6  | 87.6  | 88.9  | 84.8  |
| Total solids   | 18.2                 | 18.9                           | 16.8                   | 16.0  | 11.7  | 12.4  | 11.4  | 11.1  | 13.2  |
| Fat  | 2.0                  | 2.7                            | 3.3                    | 4.2   | 4.1   | 2.4   | 3.1   | 2.2   | 4.4   |
| Total protein (N × 6.38)   | 12.8                 | 12.5                           | 9.6                    | 8.4   | 6.0   | 5.6   | 4.7   | 4.4   | 4.0   |
| Casella (N × 6.38)   | 4.0                  | 3.0                            | 4.0                    | 4.0   | 3.6   | 3.4   | 3.4   | 3.5   | 3.4   |
| Albumin  | 2.6                  | 2.6                            | 2.2                    | 1.9   | 1.7   | 1.1   | 0.6   | 0.4   | 0.2   |
| Globulin   | 6.2                  | 6.0                            | 3.4                    | 2.5   | 1.3   | 1.1   | 0.7   | 0.5   | 0.4   |
| Lactose  | 2.2                  | 2.5                            | 2.7                    | 2.8   | 2.0   | 2.4   | 2.5   | 2.7   | 3.0   |
| Ash  | 0.9                  | 0.9                            | 0.9                    | 0.9   | 0.9   | 0.9   | 0.8   | 0.8   | 0.8   |
| Coag. on boiling   | —                    | +                              | +                      | —     | —     | —     | —     | —     | —     |
| Coag. with rennet  | —                    | —                              | —                      | —     | —     | —     | +     | +     | +     |

## OBSERVATIONS

From Tables I and II\*, it can be observed that the specific gravity of chloride and total solids are higher in buffaloes' than in cows' colostrum, and that they decrease in both cases during the progressive change into milk. Though the acidity and ash content of buffaloes' colostrum are higher than those of cows' colostrum soon after calving, they decrease more rapidly, and in normal milk they are slightly lower than in the case of the cow. The fat percentage of the colostrum of both the animals just after calving is very low and the increase is not marked by any regularity; an observation which has also been recorded by other workers [ Rogers, 1935 ; Davies, 1936 and Richmond, 1930 ]. Buffaloes' colostrum is, however, richer in fat than cows' colostrum. The lactose content in both the breeds is very low at the time of calving and it rises steadily as the colostrum changes to normal milk. It is, however, observed that buffaloes' colostrum contains a lower percentage of lactose than cows' colostrum.

\* Table I represents the average of Murrah buffaloes Nos. 60, 63 and 64; and Table II represents the average of Sindhi cows Nos. 257, 261 and 275.

The high percentage of total protein in the earliest stages of the colostral period is due mainly to the high globulin content. Buffaloes' colostrum shows a higher content of total protein than cows' colostrum, and in both cases there is a fall which is more rapid during the first twenty-four hours after parturition than the following days. Both casein and albumin behave similarly in that they are present in large amounts at the time of calving, and subsequently show a gradual fall. In these constituents, also buffaloes' colostrum is richer than cows' colostrum.

The important part which globulin plays in the transmission of antibodies from the mother to her young justifies the separate analysis of this protein, which has not so far been done in previous investigations on the colostrum of bovines. Starting at the high figure of 10·9 per cent in buffaloes and 6·2 per cent in cows, it falls abruptly within the first twenty-four hours by about 75 per cent in the former and 50 per cent in the latter. The rapidity of the subsequent fall, though not as great as in the beginning, is sufficient to result in only traces of this protein fraction being left in the normal milk.

#### SUMMARY

1. Buffaloes' colostrum is higher in specific gravity, acidity, sodium chloride, total solids, total protein, casein, albumin, globulin and ash than cows' colostrum, while in lactose it is lower.
2. In both animals there is a progressive fall in all the constituents except lactose which shows an increase and fat which is irregular.
3. Globulin constitutes the major part of the total protein of both kinds of colostrum soon after calving, and its decrease is more marked during the first twenty-four hours after parturition than during the remainder of the colostral period.
4. Buffaloes' colostrum changes to normal milk in about three days, while cows' colostrum takes about four days.

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# THE INCIDENCE OF *SALMONELLA ENTERITIDIS* VAR *DUBLIN* IN PYOSEPTICAEMIA OF CALVES IN INDIA\*

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THE writer is indebted to Mr. Shirlaw for the material which forms the subject of this determinative study. The culture was received in the form of two agar-slants with a request to have it typed, and it was followed with the following history :—"This is an organism which has recently been isolated from a specific enteritis of calves, and the work which we have done so far in this laboratory, up-to-date, would seem to indicate that this organism is of primary pathological importance in this disease". The pathology of this disease has been recorded by Shirlaw [1935]. He has also sent a further note [Shirlaw, 1936] on the identity of the organism, based on our report.

On receipt, the organism was put to a complete morphological and cultural test. The result was as follows: *Morphology* (in nutrient broth after 24 hours incubation at 37°C.) :—Short Gram-negative rods, 0·9 to 1·6  $\mu$  in length, and 0·6 to 0·7  $\mu$  in breadth, with parallel or slightly bulging sides. Their ends are rounded and axis straight. They are arranged in singles and occasionally in pairs. Endospores and capsules are not formed. They are actively motile by means of peritrichiate flagella. Stained by Leishman-stain, most of the elements take a remarkably bipolar staining.

*Description of Single Colonies.*—Grown on plain-agar plates at 37°C. for 24 hours, colonies are circular in shape with a smooth surface and an entire margin. They measure 1·5 to 2 mm. in diameter. They are low-convex and are easily emulsified forming a homogeneous emulsion when rubbed up with a drop of water. They are butterous in consistency, are glistening and are transparent.

*Description of growth on slants.*—Grown on plain-agar slants at 37°C. for 48 hours, a moderate slightly spreading growth takes place. The edges are slightly spreading and undulate. Its surface is moist and has a beaten-copper appearance over the tract of the needle and a somewhat scaly appearance over the spreading margin. On old agar-slants the surface has an oily sheen, but there is no iridescence.

*Slab growth in gelatin.*—Growth along needle tract.

On potato, growth is moderately rich and has no colour.

It is an aerobe and a facultative anaerobe.

\* Paper read at the Indian Science Congress, Calcutta, 1938.

*Fermentation of Carbohydrates.*—The following are fermented with the production of acid and gas:—Dextrose, maltose, mannositol, xylose, dulsitol, dextrin, sorbitol, rhamnose, glucose, galactose, laevulose, mannose and trehalose. Acid is produced in starch. Lactose, sucrose, salicin, inositol, glycerol, inulin, raffinose, adenine and arabinose are not attacked.

*Other biochemical reactions.*—Litmus-milk is rendered slightly acid in 24 hours, but later becomes alkaline. Tested for Indol in 1 per cent peptone water after 5 days' growth, using Bohme's reagents, a faint colour appears which disappears with time. It is negative for the Voges-Proskauer test, but positive for the methyl-red test. It reduces nitrates, produces hydrogen-sulphide and is positive for catalase and methylene blue reductase.

The morphological, cultural and biochemical characters are typical of the salmonella group and resemble those of *S. enteritidis*, *S. newport*, *S. dar-es-salaam*, *S. stanley* and certain other members of the salmonella group. A preliminary agglutination test was undertaken to ascertain which of the various heat-stable somatic antigens occurring in the salmonella group was concerned in the organism under study.

The serum for this agglutination test was obtained as follows: the Shirlaw's organism was grown in plain agar in a Petri-dish for 24 hours. It was then washed with normal saline solution containing 0.1 per cent of formalin. It was then adjusted to an opacity of 300 million bacteria per c.c. with saline containing 0.1 per cent of formalin. This emulsion was injected intravenously, after a storage of 3 days at room temperature, into a rabbit five times at intervals of five to six days. The doses for the successive injections were 0.1 c.c., 0.5 c.c. for the first and second injections respectively, and 1 c.c. for the subsequent three injections. The rabbit was bled for serum on the ninth day after the last injection. Its titre then was over 12,000, the end titre not having been determined.

The heat-stable antigens were made according to the technique of Bruce-White [1926]. Roux flasks containing agar with a 1:800 concentration of phenol, were sown with the selected cultures. After an incubation of 48 hours, the cultures were washed in 0.1 per cent saline and heated in a water-bath at the boiling point for about thirty to forty minutes. The opacity of the emulsions was then made up to 200 million bacteria per c.c. and formalin was added to give a concentration of 0.1 per cent.

The serum was diluted in series in such a manner, that, when an equal quantity of the bacterial emulsion was added, the final dilution of the serum in the successive tubes became 25, 50, 100, 200, 400, and 800 respectively.

The strains were selected for this test so that all the somatic factors then known to occur in the salmonella group were represented.

The test was conducted in the water-bath at 55°C. and readings were taken at the end of four hours and twenty-four hours. The results are tabulated in Table No. I.

TABLE I

| Phenol-agar heated<br>antigens | Source<br>Nat. Inst. Type<br>Cultures<br>No. | Heat-stable<br>Somatic Factors | Titre   |          |
|--------------------------------|--|--------------------------------|---------|----------|
|                                |  |                                | 4 hours | 24 hours |
| <i>S. paratyphi-A</i>          | 13   | I, II                          | Nil     | Nil      |
| <i>S. paratyphi-B</i>          | 15   | IV, V (7, 8)                   | Nil     | 200      |
| <i>S. abortus-equi</i>         | 766  | IV (7, 8)                      | 400     | 800      |
| <i>S. paratyphi-C</i>          | 90   | VI, VII                        | Nil     | Nil      |
| <i>S. stanley</i>              | 92   | IV, V (7, 8)                   | 50      | 200      |
| <i>S. reading</i>              | 72   | IV (7, 8)                      | Nil     | 100      |
| <i>S. derby</i>                | 1,729  | IV (7, 8)                      | 200     | 400      |
| <i>S. typhi</i>                | 786  | IX (8)                         | *800    | *800     |
| <i>S. enteritidis</i> †        | 410  | IX (8)                         | Nil     | 100      |
| <i>S. pullorum</i>             | 969  | IX                             | *800    | *800     |
| <i>S. gallinarum</i>           | 416  | IX                             | *800    | *800     |
| <i>S. anatum</i>               | Local Strain                                 | X, III                         | Nil     | Nil      |
| <i>S. newport</i>              | 129  | VI, VIII (7)                   | Nil     | 100      |
| Shirlaw's organism             | Isolated from<br>calves at<br>Lahore.        | ?                              | 400     | *800     |

\* End titre not determined.

† This culture which has been in subculture in this laboratory for the last fifteen years has since been found, by appropriate tests, to be in the "O" and "R" form.

(The Roman figures represent the somatic antigenic structures suggested by the Salmonella Sub-Committee of the International Society for Microbiology, and the Arabic figures within brackets represent additional somatic factors recognised by Bruce-White during 1926.)

The tests were repeated with some of the organisms in which the end titre had not been reached with a view to find out the end titre. The results were as follows :—

|                        |   |   |   |   |                 |
|------------------------|---|---|---|---|-----------------|
| <i>S. abortus-equi</i> | . | . | . | . | End titre 6,400 |
| <i>S. typhi</i>        | . | . | . | . | " 6,400         |
| <i>S. gallinarum</i>   | . | . | . | . | " 6,400         |

The above tests show that the somatic antigen of the organism under study is remarkably related to that of *S. pullorum*, *S. typhi*, *S. gallinarum* and *S. abortus-equi* and to a lesser extent to *S. stanley*, *S. derby*, *S. reading*, *S. paratyphi-B*, *S. newport* and *S. enteritidis* "410".

The strong relationship to *pullorum* and *gallinarum*, organisms which possess each a single somatic factor namely IX of the Kauffmann-White schema, strongly suggests that factor IX is at least one of the components of the somatic antigen of the Shirlaw's organism. It is also remarkably related to *S. abortus-equi*. The only somatic antigenic factor that it contains, according to the Kauffmann-White schema, is factor IV. The following absorption test was, therefore, conducted to verify if factor IV was also concerned in this organism.

Whole serum prepared against Shirlaw's organism was put to agglutination test against phenol-agar-heated emulsions of certain strains containing factor IX and other strains containing factor IV. Portions of this serum were then absorbed with O-antigens of these several strains, and the absorbed sera again put to test against the same set of organisms as in the first instance. The result is tabulated in Table II below.

TABLE II

| Agglutination conducted against phenol-agar-heated emulsions. | Titre of unabsorbed serum | Serum made against Shirlaw's organism homologous titre 12,800 |                 |                        |                   |                 |                       |                   |
|---|---------------------------|---|-----------------|------------------------|-------------------|-----------------|-----------------------|-------------------|
|   |                           | Titre after absorption with phenol-agar-heated emulsions of   |                 |                        |                   |                 |                       |                   |
|   |                           | <i>S. pullorum</i>  | <i>S. typhi</i> | <i>S. abortus-equi</i> | <i>S. stanley</i> | <i>S. derby</i> | <i>S. paratyphi-B</i> | <i>S. reading</i> |
| Shirlaw's organism . . .                                      | 6,400                     | <100  | <100            | 400                    | 200               | 200             | 400                   | 200               |
| <i>S. pullorum</i> . . .                                      | 6,400                     | <100  | <100            | 800                    | 1,600             | 800             | 1,600                 | 800               |
| <i>S. abortus-equi</i> . . .                                  | 6,400                     | <100  | <100            | 100                    | 100               | <100            | <100                  | <100              |
| <i>S. typhi</i> . . .   | 6,400                     | <100  | <100            | 800                    | 1,600             | 800             | 1,600                 | 800               |
| <i>S. derby</i> . . .   | 100                       | 0   | 0               | <100                   | 100               | <100            | 100                   | 0                 |
| <i>S. stanley</i> . . .                                       | 50                        | 0   | 0               | 0                      | <100              | 0               | 0                     | 0                 |
| <i>S. reading</i> . . .                                       | 50                        | 0   | 0               | 0                      | 0                 | 0               | 0                     | <100              |
| <i>S. paratyphi-B</i> . . .                                   | 100                       | 0   | 0               | 0                      | 0                 | 0               | <100                  | 0                 |

NOTE.— { 0 = Test not conducted.  
The lowest titre tested after absorption was 100.

A second absorption test was done in a modified way to verify the above results with the result shown in Table III.

TABLE III

| Antigen                    | Shirlaw's whole serum |         |                       |         |                   |         |
|----------------------------|-----------------------|---------|-----------------------|---------|-------------------|---------|
|                            | Before absorp-        |         | After absorption with |         |                   |         |
|                            |                       |         | <i>S. pullorum</i>    |         | <i>S. stanley</i> |         |
|                            | 4 hrs.                | 18 hrs. | 4 hrs.                | 18 hrs. | 4 hrs.            | 18 hrs. |
| Shirlaw's organism whole . | 6,400                 | 12,800  | 3,200                 | 6,400   | 3,200             | 3,200   |
| Shirlaw's organism "O" .   | 400                   | 1,000   | <50                   | <50     | <50               | <50     |
| <i>S. pullorum</i> .       | 1,600                 | 1,000   | <50                   | <50     | <50               | <50     |
| <i>S. abortus-equi</i> .   | 400                   | 1,000   | 50                    | <50     | <50               | <50     |
| <i>S. derby</i> , whole .  | 1,600                 | 3,200   | 800                   | 1,000   | 800               | 1,000   |
| <i>S. derby</i> , "O" .    | 50                    | 200     | <50                   | <50     | <50               | <50     |

NOTE.—The lowest titre tested was 50.

It will be seen that *S. pullorum* and *S. typhi* divest Shirlaw's serum of all the somatic agglutinins for Shirlaw's organism as well as for *S. abortus-equi* and related organisms. *S. abortus-equi*, *S. stanley*, *S. derby*, *S. paratyphi-B* and *S. reading*, on the other hand, could not divest Shirlaw's serum of agglutinins for *S. pullorum*, *S. typhi*, and the Shirlaw's organism, although titres for them are much reduced (in the case of Shirlaw's organism to a remarkable extent).

This shows that the somatic antigen of Shirlaw's organism is identical with that of *S. pullorum* and *S. typhi*, and that Shirlaw's organism in common with *S. pullorum* and *S. typhi*, possess an additional factor that is also common to *S. abortus-equi* and the other antigenically related organisms enumerated above. The Kauffmann-White schema does not, however, show any common factor between *pullorum* and *typhi* on the one hand and *abortus-equi* on the other. This test seems to substantiate the existence of the common factor "8" described by Bruce-White [1926]. But as the Salmonella Sub-Committee has since accepted the Kauffmann scheme which gives no place for this factor "8", having made the above remarks, one remains content by stressing the fact that the somatic antigen of Shirlaw's organism is identical with that of *S. pullorum* (i.e., that its somatic antigen is "IX").

*Examination for Flagellar Factors.*—Having ascertained, adopting Andrew's technique [1922], that the Shirlaw's organism does not occur in the group phase, one proceeded to analyse, systematically, the specific flagellar factors of the organism under study.

For these tests, serum containing only flagellar agglutinins, to the exclusion of all somatic ones, was obtained in the following manner:—Fifteen Roux flasks containing phenol-agar were sown with the Shirlaw's organism.

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After an incubation of 48 hours, the growth was washed with formal-saline. The emulsion was centrifuged at top speed for one hour and the supernatant fluid discarded. To the deposit was added 25 c.c. of a 1 in 12·5 dilution of whole Shirlaw's serum. Absorption took place in the water-bath at 55° C. for 24 hours. The emulsion was centrifuged, and the supernatant serum recovered. This fluid was submitted to two more absorptions in the same manner.

The antigens for the agglutination tests with the absorbed serum were made as follows : The selected organisms (see table below) were grown on plain agar slants for 24 hours, at the end of which they were washed in formal-saline. Each of the emulsions was then adjusted, by addition of formal-saline to contain about 300 million organisms per c.c.

The result of this agglutination test is tabulated in Table IV.

TABLE IV  
Serum Shirlaw "H" devoid of all "O" agglutinins prepared as described above

| Serial No. | Whole antigen made as described above   | Nat. Collection Type Cultures | Flagellar factors concerned |            | Titre |
|------------|---|-------------------------------|-----------------------------|------------|-------|
|            |   |                               | Specific                    | Group      |       |
| 1          | <i>S. paratyphi-A</i>   | 13                            | a.                          | —          | <25   |
| 2          | <i>S. paratyphi-B</i>   | 156                           | b                           | 1, 2       | <25   |
| 3          | <i>S. abortus-ovis</i>  | 3,293                         | c                           | 1, 4, 6    | <25   |
| 4          | <i>S. stanley</i>   | 92                            | d                           | 1, 2       | <25   |
| 5          | <i>S. abortus-equus</i>   | 766                           | e, n, x                     | —          | <25   |
| 6          | <i>S. derby</i>   | 1,729                         | f, g                        | —          | 1,600 |
| 7          | <i>S. seftenberg</i>  | 4,165                         | g, s                        | —          | 100   |
| 8          | <i>S. reading</i>   | 72                            | h, e                        | 1, 4, 5    | <25   |
| 9          | <i>S. typhimurium</i>   | 74                            | i                           | 1, 2, 3    | <25   |
| 10         | <i>S. thompson</i>  | 2,253                         | k                           | 1, 3, 4, 5 | <25   |
| 11         | <i>S. panama</i>  | 4,221                         | l, v                        | 1, 3, 4, 5 | <25   |
| 12         | <i>S. dorcas-salama</i>   | 2,206                         | e, n, l, v                  | —          | <25   |
| 13         | <i>S. oranienberg</i>   | 3,746                         | m, t                        | —          | <25   |
| 14         | <i>S. enteritidis</i> , var.<br><i>darey</i>  | 205                           | o, g, m                     | —          | 3,200 |
| 15         | <i>S. enteritidis</i> , var.<br><i>dublin</i>   | 126                           | p, g                        | —          | 6,400 |
| 16         | <i>S. enteritidis</i> , var.<br><i>moscow</i>   | 3,205                         | q, g, o                     | —          | 1,600 |
| 17         | <i>S. virchow</i>   | 3,748                         | r                           | 1, 2, 3    | <25   |
| 18         | <i>S. enteritidis</i> , var.<br><i>rostock</i>  | 3,747                         | g, p, w                     | —          | 6,400 |
| 19         | <i>S. bareilly</i>  | 3,213-A                       | y                           | —          | <25   |
| 20         | Shirlaw's organism<br>"O" emulsion as control for completeness of absorption of "O" agglutinins | —                             | —                           | —          | 25    |

NOTE.—The lowest titre tested was 25.

It will be seen from the above that the flagellar factors involved in Shirlaw's organism must be one or more of the factors occurring in items 6, 7, 14, 16 and 18. That is, one or more of the factors f, g, s, o, p, q and w may be concerned. It is clear that a, b, c, d, e, h, i, k, l, m, n, r, t, v, x and y are not concerned. With a view to determine which of the seven factors enumerated above are involved in Shirlaw's organism, the following absorption test was performed.

TABLE V

| Antigens 24 hours' agar cultures in formal-saline | Titre before absorption | Shirlaw's whole serum       |                                   |   |   |   |  |                                 |
|---|-------------------------|-----------------------------|-----------------------------------|---|---|---|--|---------------------------------|
|   |                         | Titre after absorption with |                                   |   |   |   |  |                                 |
|   |                         | <i>S. derby</i> IV : f,g.   | <i>S. seftenberg</i> I, II : g,s. | <i>S. enteritidis</i> var. <i>dansys</i><br>IX : g,o,m. | <i>S. enteritidis</i> , var. <i>moscow</i><br>IX : a,o,q. | <i>S. enteritidis</i> var. <i>dublin</i><br>IX : g,p. | <i>S. enteritidis</i> var. <i>rostock</i><br>IX : g,p,w. | Shirlaw's<br>organism<br>IX : p |
| <i>S. derby</i>                                   | 800                     | 50                          | 50                                | 50  | 50  | 50  | 50   | 50                              |
| <i>S. seftenberg</i>                              | 400                     | 50                          | 50                                | < 50  | 50  | 50  | 50   | 50                              |
| <i>S. enteritidis</i> var. <i>dansys</i>          | 6,400                   | *800                        | 800                               | 50  | < 50  | < 50  | < 50   | 50                              |
| <i>S. enteritidis</i> var. <i>moscow</i>          | 6,400                   | *800                        | *800                              | 50  | 50  | < 50  | < 50   | 50                              |
| <i>S. enteritidis</i> var. <i>dublin</i>          | 12,800                  | *800                        | *800                              | *800  | *800  | < 50  | < 50   | 50                              |
| <i>S. enteritidis</i> var. <i>rostock</i>         | 6,400                   | *800                        | *800                              | *800  | *800  | < 50  | < 50   | 50                              |
| Shirlaw's organism                                | *12,800                 | *800                        | *800                              | *800  | *800  | < 50  | < 50   | 50                              |

\* End titre not determined.

In addition, the following absorption tests were done with the results shown :—

- (1) Shirlaw-serum absorbed by *derby* leaves no agglutinins for *derby* and *seftenberg*.
- (2) Shirlaw-serum absorbed by *seftenberg* leaves no agglutinins for *derby* and *seftenberg*.
- (3) Shirlaw-serum absorbed with *enteritidis* var. *dansys* leaves no agglutinins for *derby*, *seftenberg*, *enteritidis* var. *dansys* and *enteritidis* var. *moscow*, but leaves agglutinins for *enteritidis* var. *dublin*, *enteritidis* var. *rostock* and Shirlaw-organism. This serum when further absorbed with *enteritidis* var. *dublin*, loses its residual titre for Dublin, Rostock and Shirlaw.

These tests conclusively show that g and p are the only flagellar factors that are contained in the Shirlaw-organism. By reference to the Kauffmann-White schema, the only organism with "IX" somatic antigen and "g" and "p" specific flagellar antigen is *S. enteritidis* var. *dublin*. A cross absorption test was conducted to prove the identity of Shirlaw's organism with this organism.

TABLE VI  
*Serum Shirlaw*

| Antigen  | Titre before absorption | Titre after absorption with |  |
|--|-------------------------|-----------------------------|--|
|  |                         | Shirlaw-organism            | <i>S. enteritidis</i> var. <i>dublin</i> |
| Shirlaw-organism                               | 12,000                  | Nil                         | Nil                                      |
| <i>S. enteritidis</i> var. <i>dublin</i>       | 12,000                  | Nil                         | Nil                                      |
| Serum <i>S. enteritidis</i> var. <i>dublin</i> |                         |                             |  |
| Shirlaw-organism                               | 12,000                  | Nil                         | Nil                                      |
| <i>S. enteritidis</i> var. <i>dublin</i>       | 12,000                  | Nil                         | Nil                                      |

The organism is, therefore, serologically identical with *S. enteritidis* var. *dublin*, its antigenic constitution being IX : g, p : -.

#### SUMMARY

An organism isolated from cases of pyosepticæmia in calves by Mr. Shirlaw at Lahore, has been typed as *Salmonella enteritidis* var. *dublin*, after a detailed study of its morphological, cultural and biochemical properties, as also of its antigenic constituents. Its antigenic structure has been found to be IX : g, p : - by a series of serological analysis. This is the first time the incidence of this organism is recorded in India.

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DETERMINATION OF SEX FROM THE EXTERNAL  
FEATURES OF *LABEO ROHITA*, GÜNTHER AND  
*CIRRHINA MRIGALA* CUV. AND VAL.

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In connection with the scheme of fresh-water fishes of Bengal which we are tackling in the Zoology Department of the University of Calcutta, and partly financed by the Imperial Council of Agricultural Research, India, we were in great difficulty in determining the sex of two best food-fishes of Bengal, viz., *Labeo rohita* and *Cirrhina mrigala*. On consulting the literature we could not find any record regarding this point on these fishes of India.

We made a considerable study on the subject and found the following points which will not only be of immense use to people trained in scientific line, but also will be of equal use to the lay public which is the main idea of the scheme itself.

Regarding *Labeo rohita* it can be said that when the pectoral fin is greater than or equal to anal, it will be a male specimen. When the pectoral fin is less than the anal, it will be a female. In breeding season the area surrounding the vent of the female becomes reddish and swollen. Regarding *Cirrhina mrigala*, it can be said that when the pectoral fin is greater than the anal, it will be a male specimen like *Labeo rohita*, but when the pectoral fin is equal to anal, it will be female.

The utility of the problem is obvious as one ought to know the sex of the specimens in breeding, rearing and particularly for artificial fecundation.

In conclusion the writer wishes to acknowledge the help that he has received from his Research Assistants.



# A STUDY OF THE MINERAL ASSIMILATION OF GROWING CALVES

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IT is well known that, in feeding cattle, in addition to an adequate supply of proteins, fats and carbohydrates, minerals have to be provided in sufficient quantities and in suitable proportions. Of these phosphorus and lime play a very important part. The deficiency of these in the diet is known to cause many diseases. Orr [1929] in his monograph on "Minerals in Pastures," Crichton [1930] on "Mineral requirements of Dairy Cattle" and Theiler and Green [1931] in their paper on "Aphosphorosis in ruminants" have given copious references to literature on this subject and have recorded many cases where beneficial results have accrued by feeding suitable mineral supplements.

Work of this nature in India is of recent growth and indications are not wanting that the feeds in many parts of India are definitely deficient in one or more mineral ingredients which may be one of the causes of stunted growth and emaciated appearance of the animals in those areas. Chaudhari [1933] has reported that calves which were stunted in growth, improved considerably in weight and condition after being fed a mineral supplement.

The present paper is a study of the mineral assimilation of growing calves, especially of calcium and phosphorus under normal conditions of feeding obtained at the Bangalore Dairy Farm.

## EXPERIMENTAL

Eight bull calves, 14 to 17 months old, were selected and divided into two groups of four each in such a way that the average weight and age of the animals in each group remained approximately the same. Group I received the basal ration which consisted of hay, Guinea grass (green), wheat bran and groundnut cake. The quantities of concentrates provided were so adjusted as to admit of a liberal amount of protein for maintenance and growth. This hay was particularly selected as it was known to be poor in phosphoric acid, where the average percentage was only 0.052. Group II received the same

basal ration supplemented by 30 grm. of calcium phosphate, which raised both the calcium and phosphorus content of the ration. The hay was fed *ad lib* and the daily consumption of each animal was determined accurately. Guinea grass, which was added to meet the vitamin requirements, was given at the rate of one kg. per head per day irrespective of age or weight. The animals were weighed every day and according to the average weight at the end of the week were given increased quantities of concentrate to meet the increased demands of protein due to growth. Before the mineral feeding was commenced, a digestion and mineral balance experiment with all the animals was carried out. (Experiment I). After seventeen weeks, another digestion and mineral balance experiment was carried out (Experiment II) and then the animals were reversed, that is to say, Group I which was getting no mineral now received the mineral supplement and Group II which originally received the mineral supplement was deprived of it. The experiment was continued for a further twelve weeks, at the end of which a third digestion and mineral balance experiment was conducted (Experiment III).

TABLE I  
*Analysis of foodstuffs*

(Percentage on dry basis)

| Foodstuff:      | Total N | Alb. N | Ash   | Silica | Ash Sol. in HCl. | Ether extract | Crude fibre |
|-----------------|---------|--------|-------|--------|------------------|---------------|-------------|
| Experiment I    |         |        |       |        |                  |               |             |
| Hay . . .       | 0.307   | 0.266  | 14.62 | 11.78  | 2.84             | 1.489         | 36.96       |
| Guinea Grass    | 1.163   | 0.857  | 12.15 | 4.73   | 7.42             | 2.198         | 40.06       |
| Groundnut cake. | 8.598   | 8.152  | 5.92  | 1.16   | 4.76             | 9.728         | 5.732       |
| Wheat bran .    | 2.154   | 1.963  | 5.24  | 0.98   | 4.26             | 2.326         | 11.11       |
| Experiment II   |         |        |       |        |                  |               |             |
| Hay . . .       | 0.271   | 0.212  | 11.04 | 8.81   | 2.23             | 1.854         | 37.23       |
| Guinea Grass    | 1.083   | 0.752  | 10.90 | 4.46   | 6.44             | 1.723         | 42.85       |
| Groundnut cake. | 8.508   | 8.136  | 5.18  | 1.20   | 3.98             | 8.895         | 6.089       |
| Wheat bran .    | 2.134   | 1.864  | 10.98 | 6.37   | 4.61             | 3.417         | 13.78       |
| Experiment III  |         |        |       |        |                  |               |             |
| Hay . . .       | 0.379   | 0.289  | 11.77 | 9.56   | 2.21             | 2.088         | 37.05       |
| Guinea grass    | 1.502   | 1.091  | 13.02 | 5.40   | 7.62             | 2.396         | 36.53       |
| Groundnut cake. | 7.973   | 7.912  | 4.77  | 1.04   | 3.73             | 8.009         | 7.120       |
| Wheat bran .    | 2.000   | 1.801  | 8.82  | 4.29   | 4.53             | 2.940         | 14.65       |

TABLE II

*Mineral composition of the foodstuffs*

(Percentage on dry basis)

|                   | P <sub>2</sub> O <sub>5</sub> | CaO   | MgO   | Na <sub>2</sub> O | K <sub>2</sub> O |
|-------------------|-------------------------------|-------|-------|-------------------|------------------|
| Experiment I      | Hay . . .                     | 0·000 | 0·845 | 0·300             | 0·322            |
|                   | Guinea grass . . .            | 0·519 | 0·765 | 0·482             | 0·348            |
|                   | Wheat bran . . .              | 1·577 | 0·075 | 0·553             | 0·641            |
|                   | Groundnut cake . . .          | 1·281 | 0·418 | 0·579             | 0·366            |
| Experiment II     | Hay . . .                     | 0·048 | 0·677 | 0·405             | 0·284            |
|                   | Guinea grass . . .            | 0·493 | 0·586 | 0·379             | 0·429            |
|                   | Wheat bran . . .              | 1·791 | 0·219 | 0·554             | 0·355            |
|                   | Groundnut cake . . .          | 1·188 | 0·273 | 0·485             | 0·476            |
| Experiment III    | Hay . . .                     | 0·048 | 0·646 | 0·344             | 0·241            |
|                   | Guinea grass . . .            | 0·520 | 0·842 | 0·567             | 0·229            |
|                   | Wheat bran . . .              | 1·482 | 0·265 | 0·005             | 0·222            |
|                   | Groundnut cake . . .          | 1·174 | 0·199 | 0·516             | 0·327            |
| Hay-Average . . . | 0·052                         | 0·723 | 0·370 | 0·282             | 0·519            |

## RESULTS

The chemical composition of the foodstuffs is given in Table I. It will be noticed that the hay during experiment II is of poorer quality, as judged from the figure for total nitrogen. The other figures do not call for any special remarks. The mineral composition of the foodstuffs is given in Table II and it may be mentioned here that the hay is deficient in phosphoric acid, and the actual average quantity of lime and phosphoric acid obtained from the hay consumed by each animal per day during experiment I is 24·27 grm. and 1·71 grm. respectively.

## THE DIGESTIBILITY OF THE RATION

The digestibility coefficients of the different constituents during the experiments are shown in Table III.

TABLE III

*Digestibility coefficients of experiments I, II and III.*

| Calf No.                                | Dry matter           | Organic matter | Crude Protein | Ether extract | Crude fibre | Nitrogen free extract | Total carbohydrates |       |
|---|----------------------|----------------|---------------|---------------|-------------|-----------------------|---------------------|-------|
| Experiment I<br>Without Minerals        | 51.12                | 56.26          | 73.89         | 71.00         | 51.39       | 54.86                 | 53.45               |       |
|   | 49.98                | 55.14          | 74.92         | 72.77         | 48.03       | 54.19                 | 51.72               |       |
|   | 49.55                | 54.78          | 70.83         | 63.61         | 50.20       | 53.53                 | 52.19               |       |
|   | 51.60                | 57.48          | 71.23         | 58.46         | 54.43       | 55.80                 | 55.26               |       |
|   | 51.50                | 57.33          | 74.40         | 66.82         | 51.56       | 56.07                 | 54.29               |       |
|   | 49.84                | 55.24          | 74.90         | 72.35         | 47.74       | 54.15                 | 51.61               |       |
|   | 51.77                | 56.70          | 74.01         | 60.35         | 49.11       | 57.13                 | 53.89               |       |
|   | 49.77                | 54.63          | 60.81         | 60.31         | 48.83       | 54.72                 | 52.32               |       |
| Average                                 | 50.63                | 55.93          | 73.00         | 66.83         | 50.16       | 55.06                 | 53.09               |       |
| Experiment II<br>Group I<br>No-minerals | 49.74                | 54.87          | 69.86         | 62.91         | 54.02       | 51.95                 | 52.91               |       |
|   | 49.33                | 54.14          | 67.20         | 63.70         | 51.78       | 51.73                 | 51.75               |       |
|   | 47.02                | 51.96          | 65.81         | 57.48         | 50.10       | 49.77                 | 49.90               |       |
|   | 45.51                | 50.51          | 65.52         | 58.39         | 48.48       | 48.45                 | 48.46               |       |
|   | Average              | 47.90          | 52.87         | 67.10         | 63.62       | 51.10                 | 50.48               | 50.76 |
|   | Group II<br>Minerals | 45.84          | 51.31         | 68.10         | 59.83       | 48.22                 | 49.39               | 48.92 |
|   |                      | 46.44          | 52.20         | 69.92         | 61.92       | 47.51                 | 50.88               | 49.54 |
|   |                      | 47.64          | 52.54         | 69.12         | 64.29       | 48.96                 | 50.75               | 50.03 |
|   |                      | 43.84          | 48.98         | 61.51         | 53.88       | 46.41                 | 47.99               | 47.35 |
| Average                                 | 45.94                | 51.27          | 67.16         | 59.98         | 47.78       | 49.75                 | 48.96               |       |
| Experiment III<br>Minerals              | 48.04                | 53.90          | 71.00         | 59.34         | 52.03       | 51.38                 | 51.64               |       |
|   | 53.60                | 58.53          | 74.24         | 64.91         | 56.19       | 56.38                 | 56.31               |       |
|   | 52.81                | 58.48          | 73.65         | 59.32         | 56.43       | 56.33                 | 56.37               |       |
|   | 51.83                | 56.82          | 72.31         | 62.55         | 54.82       | 54.60                 | 54.69               |       |
|   | Average              | 51.59          | 56.93         | 72.80         | 61.53       | 54.87                 | 54.67               | 54.75 |
|   | No-minerals          | 49.36          | 54.01         | 72.15         | 60.63       | 51.03                 | 52.00               | 51.61 |
|   |                      | 50.85          | 56.23         | 74.01         | 61.11       | 54.04                 | 53.32               | 53.61 |
|   |                      | 52.51          | 56.91         | 74.12         | 60.20       | 55.88                 | 53.75               | 54.60 |
|   |                      | 49.91          | 58.99         | 71.63         | 62.69       | 57.73                 | 56.89               | 57.23 |
| Average                                 | 50.66                | 56.54          | 72.98         | 61.16         | 54.67       | 53.99                 | 54.26               |       |

A summary of these is given in Table IV.

From these figures it may be observed that the digestibility during experiment II is, as compared with experiments I & III, slightly lower. This is due to the poor quality of the hay fed during this experiment. Between the two groups, during experiments II & III, the digestibility of the several ingredients remain practically the same. The digestibility of the crude protein of the mineral and no-mineral groups is 67·10 and 67·16 in experiment II and 72·80 and 72·98 in experiment III and this agreement is remarkable, in spite of the fact, that one set of animals in each experiment received an increased quantity of phosphoric acid in the ration.

TABLE IV

*Summary of the average digestion coefficients of experiments I, II and III*

|                                       | Dry matter | Organic matter | Crude Protein | Ether extract | Crude fibre | Nitrogen free extract | Total carbohydrates |
|---------------------------------------|------------|----------------|---------------|---------------|-------------|-----------------------|---------------------|
| Experiment I                          | 50·63      | 55·93          | 73·00         | 66·83         | 50·16       | 55·06                 | 53·09               |
| Experiment II<br>Group I, No-minerals | 47·90      | 52·87          | 67·10         | 60·62         | 51·10       | 50·48                 | 50·76               |
| Group II,<br>Minerals                 | 45·94      | 51·27          | 67·16         | 50·98         | 47·78       | 49·75                 | 48·96               |
| Experiment III<br>Minerals            | 51·59      | 56·93          | 72·80         | 61·53         | 54·87       | 54·67                 | 54·75               |
| No-minerals                           | 50·66      | 56·54          | 72·98         | 61·16         | 54·67       | 53·99                 | 54·26               |

## FOOD CONSUMPTION AND LIVE-WEIGHT INCREASE

The food consumption of the calves was considered satisfactory and it steadily increased week by week with the growth of the animals. The initial live-weight and the increase in weight in lb. of each animal on a thousand lb. basis for every week during the course of the experiment are given in Table V.

TABLE V

*Live-weight increase of calves per week on 1,000 lb. basis*

| Weeks              | Group A. No-Minerals |      |      |      |         | Group B. with Minerals |      |      |      |         | Remarks |  |
|--------------------|----------------------|------|------|------|---------|------------------------|------|------|------|---------|---------|--|
|                    | Calf No.             |      |      |      |         | Calf No.               |      |      |      |         |         |  |
|                    | 1                    | 2    | 3    | 4    | Average | 5                      | 6    | 7    | 8    | Average |         |  |
| <i>1st Period</i>  |                      |      |      |      |         |                        |      |      |      |         |         |  |
| 4th May 1935       | .                    | 418  | 368  | 261  | 247     | 323.5                  | 380  | 333  | 345  | 209     | 316.5   |  |
| 11th May 1935      | .                    | 45.5 | 32.0 | 42.0 | 24.0    | 35.0                   | 7.0  | 39.0 | 40.7 | 28.8    | 28.9    |  |
| 18th May 1935      | .                    | 13.9 | 15.9 | 7.2  | 4.0     | 10.2                   | 54.4 | ...  | 11.2 | 14.0    | 28.5    |  |
| 25th May 1935      | .                    | 15.9 | 11.4 | 14.7 | 4.0     | 11.5                   | 7.8  | ...  | 2.0  | 9.2     | 6.3     |  |
| 1st June 1935      | .                    | 20.0 | 15.5 | 45.4 | 23.6    | 26.1                   | 12.3 | 23.1 | 24.7 | 22.9    | 20.7    |  |
| 8th June 1935      | .                    | 17.5 | 20.2 | 5.7  | 28.5    | 19.5                   | 10.0 | 20.2 | 10.8 | 31.2    | 18.0    |  |
| 15th June 1935     | .                    | ...  | 10.2 | 10.4 | 22.3    | 14.3                   | 5.0  | 13.9 | 13.3 | ...     | 10.7    |  |
| 22nd June 1935     | .                    | 15.0 | 9.0  | 15.0 | 21.0    | 15.4                   | 17.3 | 21.0 | 13.1 | 4.4     | 14.2    |  |
| 29th June 1935     | .                    | 25.4 | ...  | 18.6 | 25.2    | 21.6                   | 19.5 | 13.4 | 18.1 | 17.2    | 17.0    |  |
| 6th July 1935      | .                    | 16.3 | 19.5 | 20.0 | 27.8    | 20.9                   | 25.2 | 21.2 | 7.7  | 16.0    | 18.0    |  |
| 13th July 1935     | .                    | 16.2 | 19.0 | 16.4 | 37.0    | 22.1                   | 20.9 | 20.8 | 12.6 | 20.8    | 18.8    |  |
| 20th July 1935     | .                    | 17.9 | 11.8 | 16.1 | 22.8    | 17.1                   | 27.3 | 20.3 | 17.4 | 28.5    | 25.9    |  |
| 27th July 1935     | .                    | ...  | 18.6 | 22.1 | 6.4     | 15.7                   | 9.0  | 17.3 | 12.3 | 19.8    | 14.6    |  |
| 3rd August 1935    | .                    | ...  | 25.0 | 21.7 | 44.3    | 30.3                   | 19.8 | 17.0 | 17.0 | 20.7    | 18.6    |  |
| 10th August 1935   | .                    | 13.8 | 13.3 | 18.2 | 21.1    | 16.6                   | 13.0 | 19.0 | 14.4 | 10.1    | 16.4    |  |
| 17th August 1935   | .                    | ...  | 21.0 | 9.0  | 17.7    | 16.2                   | 16.9 | ...  | 9.3  | 18.6    | 14.9    |  |
| 24th August 1935   | .                    | 19.4 | 17.1 | 17.8 | 29.0    | 20.8                   | 14.6 | 14.0 | 11.6 | 25.7    | 16.5    |  |
| 7th September 1935 | .                    | 28.5 | 37.8 | 60.6 | 39.0    | 36.5                   | 16.5 | 29.0 | 23.0 | 22.0    | 26.1    |  |
| Average for group  |                      |      |      | 10.6 |         |                        |      | 18.5 |      |         |         |  |

TABLE V.—(contd.)

| Weeks               | 2nd Period          |      |      |      |         |                       |       |      |      |         | Initial weight. |  |
|---------------------|---------------------|------|------|------|---------|-----------------------|-------|------|------|---------|-----------------|--|
|                     | Calf No.            |      |      |      |         | Calf No.              |       |      |      |         |                 |  |
|                     | 1                   | 2    | 3    | 4    | Average | 5                     | 6     | 7    | 8    | Average |                 |  |
|                     | 582                 | 494  | 359  | 373  | 462     | 494                   | 445   | 446  | 293  | 429     |                 |  |
|                     | With Minerals.      |      |      |      |         |                       |       |      |      |         | No-Minerals     |  |
| 14th September 1935 | 11.1                | 10.0 | 14.9 | 26.0 | 15.7    | 24.3                  | 26.5  | 20.2 | 27.4 | 24.7    |                 |  |
| 21st September 1935 | 20.1                | 26.0 | 19.6 | 26.1 | 22.9    | 25.8                  | 26.1  | 6.6  | 26.7 | 21.3    |                 |  |
| 28th September 1935 | 14.3                | 13.8 | 21.4 | 20.4 | 17.5    | 13.5                  | 17.0  | 17.5 | 13.0 | 15.2    |                 |  |
| 5th October 1935    | 19.5                | 13.5 | 13.1 | ...  | 20.0    | 16.5                  | 13.5  | 16.7 | 6.5  | 6.5     |                 |  |
| 12th October 1935   | 15.2                | 13.3 | 7.8  | 14.6 | 12.7    | 11.3                  | 14.3  | 12.9 | 31.9 | 17.6    |                 |  |
| Weeks.              | Group A No-Minerals |      |      |      |         | Group B With Minerals |       |      |      |         | Remarks         |  |
|                     | Calf No.            |      |      |      |         | Calf No.              |       |      |      |         |                 |  |
|                     | 1                   | 2    | 3    | 4    | Average | 5                     | 6     | 7    | 8    | Average |                 |  |
| 19th October 1935   | ...                 | 13.1 | 23.1 | 24.0 | 20.1    | ...                   | 16.2  | 16.9 | 12.4 | 15.2    |                 |  |
| 26th October 1935   | 29.1                | 11.1 | 5.0  | 21.2 | 16.4    | 14.0                  | 22.9  | 4.1  | 3.1  | 13.7    |                 |  |
| 2nd November 1935   | 6.9                 | 7.4  | 15.0 | 13.9 | 10.8    | 7.4                   | 20.6  | 12.4 | 9.1  | 12.4    |                 |  |
| 9th November 1935   | 13.6                | 12.8 | 27.0 | 13.7 | 16.8    | 18.1                  | 16.1  | 12.9 | 9.0  | 13.9    |                 |  |
| 16th November 1935  | 16.8                | 12.6 | 16.8 | 20.2 | 16.6    | 5.3                   | 19.9  | 22.1 | 23.0 | 17.8    |                 |  |
| 23rd November 1935  | 1.8                 | 3.7  | 2.4  | 11.0 | 4.7     | 1.9                   | 7.8   | 24.0 | 8.8  | 5.6     |                 |  |
| 30th November 1935  | 28.0                | 14.1 | 23.5 | 32.6 | 24.6    | 26.6                  | 20.9  | 29.7 | 29.2 | 26.9    |                 |  |
| Final weight        | 625                 | 574  | 436  | 425  | 528     | 580                   | 585   | 529  | 353  | 497     |                 |  |
| Average for group   |                     |      | 16.3 |      |         |                       | 16.26 |      |      |         |                 |  |

The addition of a mineral supplement to the ration has not produced any significant effect on the live-weight increase of the calves of the two groups.

TABLE

*Mineral Balance*

|                | P <sub>2</sub> O <sub>5</sub> |        |       |         | CaO    |        |       |         |      |       |
|----------------|-------------------------------|--------|-------|---------|--------|--------|-------|---------|------|-------|
|                | Intake                        | Out go |       | Balance | Intake | Out go |       | Balance |      |       |
|                |                               | Faeces | Urine |         |        | Faeces | Urine |         |      |       |
| Experiment I   | Calf 1                        | 22.11  | 14.99 | 0.11    | 7.01   | 43.35  | 32.19 | 2.36    | 6.89 |       |
|                | Calf 2                        | 20.24  | 15.56 | 0.10    | 6.58   | 34.52  | 27.44 | 2.32    | 4.71 |       |
|                | Calf 3                        | 15.88  | 10.47 | 0.14    | 5.27   | 20.92  | 19.73 | 1.52    | 3.67 |       |
|                | Calf 4                        | 15.05  | 13.53 | 0.08    | 1.44   | 22.02  | 15.00 | 2.57    | 4.45 |       |
|                | Calf 5                        | 20.15  | 16.37 | 0.11    | 3.67   | 31.15  | 26.84 | 2.33    | 1.98 |       |
|                | Calf 6                        | 18.02  | 12.57 | 0.10    | 6.35   | 29.64  | 24.47 | 1.96    | 3.23 |       |
|                | Calf 7                        | 19.64  | 12.59 | 0.17    | 6.88   | 34.80  | 27.31 | 1.50    | 5.99 |       |
|                | Calf 8                        | 13.12  | 9.53  | 0.06    | 3.53   | 23.06  | 16.42 | 2.03    | 5.51 |       |
| Average        |                               | 18.15  | ...   | ...     | 5.09   | 30.56  | ...   | ...     | 6.79 |       |
| Experiment II  | No mineral                    | Calf 1 | 23.54 | 16.13   | 0.11   | 7.99   | 34.09 | 25.29   | 3.15 | 6.25  |
|                | No mineral                    | Calf 2 | 22.53 | 14.91   | 0.14   | 7.48   | 32.05 | 22.23   | 2.54 | 7.39  |
|                | No mineral                    | Calf 3 | 18.96 | 12.94   | 0.13   | 5.89   | 25.56 | 17.28   | 1.28 | 6.35  |
|                | No mineral                    | Calf 4 | 19.52 | 13.12   | 0.05   | 6.35   | 29.30 | 21.87   | 0.88 | 6.55  |
|                | Average                       | 21.14  | ...   | ...     | 6.76   | 30.40  | ...   | ...     | 6.61 |       |
|                | With mineral                  | Calf 5 | 34.27 | 26.90   | 0.13   | 8.25   | 42.18 | 32.11   | 2.30 | 7.34  |
|                | With mineral                  | Calf 6 | 33.13 | 24.87   | 0.17   | 8.30   | 37.97 | 29.08   | 1.38 | 9.51  |
|                | With mineral                  | Calf 7 | 31.60 | 25.02   | 0.15   | 8.23   | 39.32 | 27.19   | 1.91 | 11.27 |
|                | With mineral                  | Calf 8 | 35.52 | 25.75   | 0.07   | 5.79   | 34.89 | 20.68   | 2.40 | 5.89  |
| Average        |                               | 32.58  | ...   | ...     | 7.69   | 38.93  | ...   | ...     | 8.51 |       |
| Experiment III | With minerals                 | Calf 1 | 33.03 | 22.95   | 0.14   | 11.94  | 37.21 | 30.95   | 2.10 | 5.09  |
|                | With minerals                 | Calf 2 | 31.67 | 19.93   | 0.19   | 11.58  | 44.18 | 31.00   | 2.94 | 10.15 |
|                | With minerals                 | Calf 3 | 32.18 | 20.17   | 0.17   | 15.84  | 37.68 | 28.39   | 1.75 | 7.63  |
|                | With minerals                 | Calf 4 | 33.31 | 19.60   | 0.16   | 13.55  | 43.11 | 31.14   | 2.29 | 9.71  |
|                | Average                       | 33.80  | ...   | ...     | 13.98  | 43.13  | ...   | ...     | 8.23 |       |
|                | No mineral                    | Calf 5 | 24.01 | 14.59   | 0.18   | 10.19  | 38.19 | 33.56   | 4.08 | 3.59  |
|                | No mineral                    | Calf 6 | 21.81 | 13.92   | 0.10   | 7.79   | 30.84 | 27.93   | 1.56 | 3.12  |
|                | No mineral                    | Calf 7 | 22.39 | 13.84   | 0.16   | 8.35   | 33.46 | 29.12   | 1.07 | 3.27  |
|                | No mineral                    | Calf 8 | 18.30 | 11.70   | 0.06   | 6.35   | 27.65 | 25.11   | 1.58 | 2.56  |
| Average        |                               | 21.84  | ...   | ...     | 8.22   | 32.46  | ...   | ...     | 3.62 |       |

## VI

(Grm. per day)

| MgO    |        |       |         | Na <sub>2</sub> O |        |       |         | K <sub>2</sub> O |        |       |         |
|--------|--------|-------|---------|-------------------|--------|-------|---------|------------------|--------|-------|---------|
| Intake | Out go |       | Balance | Intake            | Out go |       | Balance | Intake           | Out go |       | Balance |
|        | Fees   | Urine |         |                   | Fees   | Urine |         |                  | Fees   | Urine |         |
| 23-69  | 11-10  | 8-30  | 4-11    | 35-51             | 10-80  | 3-52  | 21-10   | 46-50            | 7-88   | 35-39 | 1-32    |
| 20-25  | 10-40  | 6-42  | 3-42    | 32-84             | 12-13  | 4-64  | 16-07   | 41-35            | 6-80   | 30-59 | 3-87    |
| 15-79  | 6-61   | 5-40  | 3-78    | 28-89             | 10-26  | 6-73  | 11-90   | 33-42            | 3-59   | 25-78 | 3-65    |
| 12-50  | 5-71   | 4-92  | 1-87    | 25-69             | 10-32  | 4-44  | 11-18   | 25-87            | 3-57   | 21-25 | 2-05    |
| 15-06  | 11-84  | 5-19  | 2-03    | 31-98             | 12-55  | 6-66  | 11-77   | 30-30            | 5-50   | 33-04 | 0-67    |
| 17-90  | 7-87   | 5-58  | 4-45    | 31-30             | 12-63  | 3-53  | 15-16   | 37-73            | 3-57   | 28-32 | 5-84    |
| 20-21  | 9-08   | 5-49  | 5-66    | 33-08             | 14-42  | 4-68  | 13-98   | 30-29            | 2-83   | 32-86 | 3-60    |
| 13-77  | 5-89   | 4-01  | 3-87    | 27-76             | 10-00  | 6-58  | 11-12   | 29-35            | 2-76   | 29-37 | 4-22    |
| 17-90  | ...    | ...   | 3-65    | 30-91             | ...    | ...   | 14-05   | 26-74            | ...    | ...   | 3-15    |
| 25-99  | 12-52  | 10-74 | 2-73    | 24-56             | 15-52  | 0-48  | 18-00   | 47-56            | 12-53  | 56-91 | 8-34    |
| 23-85  | 13-23  | 9-00  | 0-72    | 33-46             | 11-33  | 2-33  | 10-80   | 44-67            | 14-19  | 24-08 | 6-10    |
| 18-98  | 9-14   | 7-70  | 2-15    | 20-09             | 11-65  | 2-58  | 15-86   | 37-25            | 8-04   | 21-25 | 7-96    |
| 21-83  | 13-47  | 3-73  | 4-63    | 31-95             | 14-80  | 0-77  | 16-28   | 40-82            | 8-29   | 25-35 | 7-18    |
| 22-67  | ...    | ...   | 2-56    | 33-62             | ...    | ...   | 17-75   | 42-63            | ...    | ...   | 7-47    |
| 24-55  | 15-10  | 8-42  | 1-04    | 33-01             | 14-01  | 1-02  | 18-08   | 46-10            | 11-58  | 59-04 | 6-72    |
| 21-63  | 12-21  | 8-46  | 0-36    | 31-89             | 12-89  | 0-51  | 18-29   | 31-47            | 12-64  | 50-82 | 8-01    |
| 22-08  | 11-28  | 7-95  | 3-75    | 32-02             | 13-90  | 0-86  | 18-07   | 43-58            | 11-00  | 24-93 | 7-45    |
| 18-64  | 9-19   | 6-81  | 2-64    | 29-65             | 11-83  | 2-61  | 15-21   | 33-47            | 12-80  | 14-49 | 8-18    |
| 21-98  | ...    | ...   | 2-10    | 33-09             | ...    | ...   | 17-46   | 41-61            | ...    | ...   | 7-59    |
| 26-64  | 15-20  | 12-08 | —1-14   | 37-10             | 13-42  | 0-87  | 22-00   | 54-07            | 18-62  | 33-71 | 1-74    |
| 25-16  | 14-43  | 9-87  | 0-86    | 36-24             | 10-23  | 1-83  | 24-38   | 53-87            | 14-10  | 24-37 | 9-31    |
| 20-92  | 12-01  | 8-69  | 0-31    | 33-42             | 7-80   | 2-60  | 22-03   | 44-21            | 9-61   | 20-59 | 3-06    |
| 21-03  | 14-50  | 9-72  | —0-16   | 35-57             | 9-81   | 0-88  | 24-88   | 49-72            | 13-62  | 24-61 | 12-00   |
| 24-20  | ...    | ...   | —0-03   | 35-61             | ...    | ...   | 23-72   | 49-97            | ...    | ...   | 6-78    |
| 26-67  | 17-52  | 9-35  | 0-20    | 37-23             | 13-13  | 1-96  | 22-12   | 54-00            | 9-95   | 59-94 | 4-11    |
| 22-62  | 13-72  | 8-63  | 0-27    | 34-51             | 9-05   | 1-15  | 23-41   | 52-83            | 9-81   | 32-03 | 0-01    |
| 24-29  | 14-61  | 8-63  | 0-06    | 35-62             | 10-22  | 1-03  | 24-37   | 50-23            | 12-19  | 35-33 | 2-71    |
| 19-02  | 12-29  | 7-49  | 0-14    | 32-91             | 8-13   | 1-29  | 23-49   | 41-66            | 14-60  | 25-80 | 1-17    |
| 23-35  | ...    | ...   | 0-29    | 35-66             | ...    | ...   | 23-55   | 48-43            | ...    | ...   | 3-5     |



## MINERAL ASSIMILATION

The mineral balance data of the different constituents for the three experiments are presented in detail in Table VI. In the three experiments the different constituents show a positive balance. The actual figures are given below :—

*Summary of mineral balance (gram. per day)*

| Experiment | P <sub>2</sub> O <sub>5</sub> | CaO  | MgO  | Na <sub>2</sub> O | K <sub>2</sub> O | Ca : P ratio of the ration (group average) |
|------------|-------------------------------|------|------|-------------------|------------------|--|
| No-mineral | 1 5.09                        | 4.79 | 3.65 | 14.05             | 3.15             | 2.76 : 1                                   |
|            | II 6.76                       | 6.61 | 2.56 | 17.75             | 7.47             | 2.35 : 1                                   |
|            | III 8.22                      | 2.63 | 0.20 | 23.35             | 3.50             | 2.43 : 1                                   |
| Mineral    | I 7.69                        | 8.51 | 2.10 | 17.46             | 7.59             | 1.96 : 1                                   |
|            | II 13.98                      | 8.22 | 0.03 | 23.72             | 6.78             | 2.09 : 1                                   |

From this it may be observed that, during the first experiment (the figures are averages for the eight animals) the assimilation of phosphoric acid and lime is positive as represented by the figures 5.09 and 4.79 respectively, the Ca : P ratio being 2.76 : 1. In experiment II with the no-mineral group, phosphoric acid and lime is positive, as in experiment I and the retention is higher, the average figures being 6.76 and 6.61 and the Ca : P ratio 2.35 : 1 and with the mineral group, consequent on the increased intake of phosphoric acid and lime, the balance for both the constituents is higher than the no-mineral group in the same experiment, the figures being 7.69 and 8.51 and the Ca : P ratio 1.96 : 1 and in experiment III, i.e. after the reversal, where the no-mineral group became the mineral group and vice versa, the mineral group showed the highest positive balance for both phosphoric acid and lime and has also retained the largest quantities, the figures being 13.98 and 8.22 and the Ca : P ratio 2.09 : 1. The figures for the no-mineral group in this experiment are 8.22 and 2.63 and the Ca : P ratio 2.43 : 1.

One other feature which is particularly noticeable is that as the lime retention increases, the magnesium retention decreases. The retention of soda has continued to increase progressively but this cannot be said of potash.

In all these experiments the resultant urine is alkaline, the pH figures being 7.87 and 8.52 for the no-mineral and the mineral group respectively in experiment II and 8.05 for both the mineral and no-mineral group in experiment III. It has been noticed that with positive phosphoric acid and lime balances the resultant urine is alkaline, as has been observed in previous experiments.

In a previous paper by one of us [Viswanatha Iyer 1935] it was shown that, when other conditions are favourable, certain minimal quantities of both phosphoric acid and lime are to be present before they yield positive balances. In these experiments the intake of both these ingredients are well above those minimal quantities.

While no difference in the appearance and the health of the animals between the mineral and no-mineral groups was noticeable, the feeding of calcium phosphate as a supplement to the animals, resulted only in a greater retention of lime and phosphoric acid in their systems. Perhaps this increased retention of lime and phosphoric acid is stored in the skeletal tissue as no additional live-weight increase was noticed corresponding to the increased mineral retention.

#### SUMMARY

From this experiment it becomes evident that when the minerals are sufficient in the feed itself, a supplement of calcium phosphate does not show any visible effect except for the fact that the animals receiving the supplement retain more calcium and phosphorus in their systems.

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## SELECTED ARTICLE

### THE NUTRITIVE VALUE OF RAW AND PASTEURIZED MILK FOR CALVES

BY

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(With 1 Figure in the Text)

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THE value of pasteurization in destroying pathogenic bacteria in milk and so safeguarding the health of the human population is beyond question. While admitting this, certain workers have hesitated to give their unreserved support to pasteurization on the ground that it may have some deleterious action on the nutritive value of the milk which will more than counteract the beneficial effect resulting from the destruction of harmful micro-organisms.

A number of papers have been published in recent years describing attempts that have been made—chiefly on rats and children—to determine whether in fact pasteurized milk is in any way inferior in its nutritive qualities to raw milk. The literature on this subject has been reviewed by Stirling and Blackwood [1933] and McCandlish and Black [1935]. Many of the results are ambiguous, and none of them, in our view, definitely answers the main question at issue. The fact that in several experiments no statistically significant difference was found between the two classes of milk is not sufficient to give us the assurance we need, for the numbers of animals or children under observation were sometimes so small as to allow a possible real difference to be obscured by the inevitable accompanying error of sampling. Moreover, the interpretation of the results is rendered difficult by the circumstance that in all these experiments the subjects under test were being fed with milk from a heterologous animal source.

It seemed to us that if a clear answer was to be obtained to our main question it was desirable to make a direct comparison of the two types of milk on the homologous animal species, namely the calf. Comparatively few experiments on this animal are on record, and those that have been described have suffered

(1) from being carried out on too small a number of animals to enable a satisfactory result to be obtained, and (2) from being complicated by the occurrence of disease in the experimental animals which may have entirely falsified the outcome of the investigation.

In planning an experiment to be carried out on calves, we determined as far as possible to avoid the sources of these errors. Since for financial and other reasons an experiment on really large numbers of animals presented formidable difficulties, it seemed of special importance to make our observations on absolutely healthy animals so that the results might not be influenced in any way by the occurrence of latent or overt disease.

#### DESCRIPTION OF EXPERIMENT

With the kind consent of the Committee of the Berks and Bucks Joint Sanatorium, permission was obtained to carry out an experiment on the herd attached to the Sanatorium. The general plan of the experiment, which lasted from July 1934 to September 1936, was to feed alternate calves, as they were born, on raw or pasteurized milk for eight weeks, and to compare their rate of growth. The relevant details may best be considered under separate headings. It may be pointed out at this stage that the management of the herd and the day-to-day control of the experiment was under the personal control of one of the authors (H. F. C.).

*The Peppard herd.*—This contains about twenty Shorthorn cows which calve down regularly throughout the year so as to maintain a more or less constant supply of milk for the Sanatorium. Except for two pedigree bulls, which have been bought in, the herd has been self-contained since 1932. The animals are kept out at pasture during both summer and winter, and are never stalled except for the actual purpose of milking, which is done by hand morning and evening. Calving takes place in the open field. Bull calves are sold at about one week old; heifer calves are mostly kept for replacements.

Since March 1925 the herd has been tested at six-monthly intervals with tuberculin; all positively reacting animals have been sold off. The last occasion on which positive reactors were found was in June 1932, when two animals had to be removed from the herd. During the whole course of the feeding experiment the herd remained completely free from tuberculosis. Moreover, at a herd test carried out a month after the conclusion of the experiment, all animals passed satisfactorily.

With regard to contagious abortion, the history of the herd has been peculiarly satisfactory. Two cows aborted in January 1928 and were found to give a positive blood agglutination reaction to *Brucella abortus*. The herd was

vaccinated the same month with a living vaccine, since when there has not been a single case of abortion. At a herd test carried out some time before the commencement of this experiment, no animals showed the presence of specific agglutinins in the blood.

John's disease has seldom occurred; the last animal to be affected with it was in September 1929.

The herd has always been relatively free from udder troubles. In October 1936, at the conclusion of the feeding experiment, the milk of each cow was examined culturally by plating in ox-blood agar, with the result that of the eighteen animals present three were found to be affected with streptococcal mastitis in a subclinical form.

*Pasteurizer.*—To ensure a supply of adequately pasteurized milk, it was decided to pasteurize the milk on the premises. A small batch pasteurizer, of 2-gallons working capacity, fitted for low-pressure steam heating, was specially manufactured for us by Messrs. Brown and Son, Ltd., London, N. 19. It was made of copper, coated with pure tin, and consisted of an outer steam-heated jacket and an inner vessel, to hold the milk, which could be lifted out for cleaning and sterilizing purposes. It was provided with an overhead motor-driven agitator and an auto-thermostatic control. In practice the milk was heated up till the indicator thermometer, which dipped into the milk itself, registered 145°F. (62.8°C.). The temperature was maintained at this level for 30 minutes, after which the steam was turned off. The milk was then cooled, either to 90°F. (32.2°C.) if it was to be fed immediately to the calves, or to the temperature of the tap water if it was to be kept for some hours.

*The milk.*—The mixed milk of the herd from the morning's milking alone was used. The required quantity was divided into two parts. One part was pasteurized at once at 145°F. (62.8°C.) for 30 minutes; the other half was left raw. Part of the raw and part of the pasteurized milk were fed at a temperature of about 90°F. (32.2°C.) to the calves in the morning. The remainder of the raw and pasteurized milk was water-cooled and kept in covered pails in a cool dairy till the evening, when it was warmed to about 90°F. and fed to the calves.

Though this was the routine practice throughout the greater part of the experiment, a misunderstanding occurred at the commencement which was responsible for some trouble during the first six months. Instead of the milk, immediately after pasteurization, being divided into two parts, one part being cooled to 90°F. and fed to the calves in the morning, the other part being cooled to tap-water temperature and kept for the evening feed, the whole of the pasteurized milk was cooled only to 90°F., and the part for the evening feed was left to cool down slowly in the dairy. As a result considerable bacterial multiplication occurred in the

pasteurized milk destined for the evening feed, and this appears to have been responsible for a certain amount of scouring in the calves. As soon as the trouble was traced to its source, the practice of leaving the pasteurized milk to cool slowly in the dairy was stopped and the routine described above was enforced. No further difficulty was experienced from scouring, except in a mild form, in occasional calves. It may be noted that the milk was always produced under cleanly conditions and was of approximately the same standard as Certified milk.

*Selection of the calves.*—In order to avoid any unconscious bias in the selection of the calves for the different groups, it was decided to allot alternate calves, as they were born, to the raw and pasteurized groups respectively\*; no selection whatsoever was made. Though this would have been an ideal method for a large experiment, it proved a little unsatisfactory in the present instance. In the first place it resulted in a very uneven distribution of the sexes, there being far fewer bull calves allocated to the pasteurized than to the raw group. In the second place it so happened that two calves, both of which at birth were quite unfit to be included in the experiment, had to be allocated to the pasteurized group. One calf, P<sub>1</sub>, was born of a mother which had to be slaughtered a week after parturition on account of "actinomycosis" of the larynx. Not only was this calf an undersized weakling at birth, but it further received a mauling by a horse when it was one day old from which it never recovered. The other, P<sub>20</sub>, had a very difficult delivery; it was unable to stand and had to be held up for feeding. The first of these animals died in three weeks, the second in a fortnight. In neither instance could death be in any way attributed to the type of diet that was being given.

These deaths, it may be noted, were the only deaths among calves that occurred throughout the course of the experiment. During the previous 15 years there had been altogether ten deaths among a total of 250 calves born. A loss of two calves during the experimental period of 27 months might therefore have been expected on the basis of the mortality rate previously experienced in the herd.

In each group there were four animals which were first calves.

*Housing of the calves.*—As soon as they were removed from their mothers, the calves were housed in a wooden shed, opening on one side into a yard about 52 × 36 ft. in area. The shed was divided into three compartments, so that the calves could be grouped more or less according to their ages. The animals in the raw and pasteurized groups were run together. Straw was used for bedding.

*The diet.*—Every calf had its mother's colostrum for the first three and a half days and was then hand-fed for eight weeks. At the beginning of the experiment the calves were given 1 lb. of milk daily per 10 lb. of body weight. This amount,

\* For the sake of convenience we ask pardon for the adjectival use, without inverted commas, of the terms raw and pasteurized in relation to the animals fed.

though more or less adequate for the first few weeks of life, was found to be insufficient for the calves as they grew older. The amount was therefore increased, though not to a sufficient extent, as it subsequently proved, to allow of optimal development. The following summarizes the diet received by the different animals:

(A) Raw calves 1—5, and pasteurized calves 2—6 were given daily 16 oz. of milk per 10 lb. of body weight for eight weeks.

(B) Raw calf 6 and pasteurized calf 7 were given daily 20 oz. of milk per 10 lb. of body weight for eight weeks.

(C) Raw calves 7—25 and pasteurized calves 8—25 (with the exception of P<sub>20</sub>) were given daily 16 oz. of milk per 10 lb. of body weight for the first four weeks, and 20 oz. of milk for the second four weeks.

The milk was fed at a temperature of about 90°F. morning and evening. During the first year of the experiment each calf was given a special feeder, from which it sucked its allotted quantity of milk through a teat. These feeders, however, proved rather troublesome, and later they were replaced by hand-feeding from a bucket.

Besides milk, the calves were provided with hay *ad lib.* Observation showed that during the first month of life they merely "played" with it, but after that each animal consumed about 1 lb. a day, rising to 3 lb. by the eighth week.

After they had completed their experimental period of observation of eight weeks, the calves that it was decided to keep in the herd were removed from the experimental shed and were given flaked maize, bran, and roots or molassed sugar-beet pulp, together with a little milk for the next few weeks.

*Recording progress.*—Each animal was weighed when it entered the experiment on the fourth day of life, and thereafter at weekly intervals. Careful observations were made on general condition, and a special watch kept for the development of rickets. From time to time outside observers, such as the milk recorder of the county and local farmers, were brought in to inspect the animals and make an attempt to distinguish those in the raw from those in the pasteurized group.

## RESULTS

The detailed results are set out in Tables I and II.

It will be noted that the average weight of animals in the raw group was 2·23 lb. greater than that of the pasteurized group at the commencement of the experiment; this was mainly the result of the smaller proportion of bull calves

in the pasteurized group. At the end of the experiment the average difference in weight between the two groups was 2·09 lb. The average increase in the animals of the raw group was 53·72 lb., and in those of the pasteurized group 53·86 lb. In other words, so far as the average weight gained throughout the whole course of the experiment was concerned, the two groups behaved for all practical purposes identically (Fig. 1).

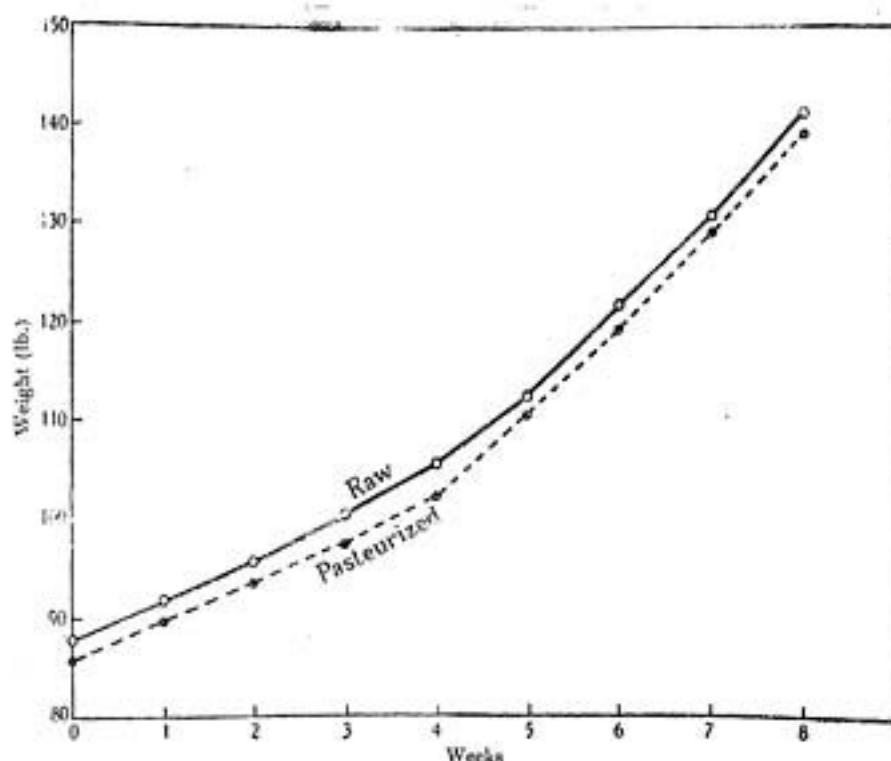


Fig. 1. Graph to show weights of all calves after 1-8 weeks.

This was all the more remarkable considering the difference in the sex distribution of the animals in the two groups. Male animals usually grow more rapidly than female. This is well shown in the raw group, in which the bull calves put on an average of 6½ lb. more weight than the heifer calves. In the pasteurized group there were approximately twice as many female as male animals and the average increase in weight of both sexes taken together might well have been expected, independent of the nature of the diet, to have been less than that in the raw group in which the sexes were more evenly distributed.

TABLE I

*Raw milk group*

| No.                           | Sex        | Weight in lb. at commencement | Weight in lb. after 8 weeks | Increase in weight in lb. | Age in months when sold | Price obtained | Remarks                             |
|-------------------------------|------------|-------------------------------|-----------------------------|---------------------------|-------------------------|----------------|-------------------------------------|
| 4                             | Bull       | 92                            | 145                         | 53                        | 10                      | 2 15           |                                     |
| 5                             | "          | 89                            | 144                         | 55                        | 10                      | 2 10           |                                     |
| 8                             | "          | 98                            | 161                         | 63                        | 10                      | 2 15           |                                     |
| 10                            | "          | 89                            | 141                         | 52                        | 10                      | 2 10           |                                     |
| 11                            | "          | 80                            | 140                         | 60                        | 10                      | 2 10           |                                     |
| 13                            | "          | 93                            | 164                         | 71                        | 10                      | 2 0            |                                     |
| 14                            | "          | 90                            | 149                         | 59                        | 10                      | 2 10           |                                     |
| 16                            | "          | 70                            | 120                         | 50                        | 10                      | 2 0            |                                     |
| 18                            | "          | 80                            | 126                         | 46                        | 10                      | 2 10           |                                     |
| 19                            | "          | 92                            | 160                         | 58                        | 10                      | 2 10           |                                     |
| 21                            | "          | 106                           | 161                         | 55                        | 10                      | 2 10           | Suckled mother for 7 days.          |
| 22                            | "          | 80                            | 141                         | 61                        | 10                      | 2 10           | Slight scouring, 5th week.          |
| 24                            | "          | 115                           | 171                         | 56                        | 2                       | 3 0            |                                     |
| 1                             | Heifer     | 90                            | 145                         | 55                        | ..                      | ..             |                                     |
| 2                             | "          | 76                            | 122                         | 46                        | 2                       | 2 4            |                                     |
| 3                             | "          | 79                            | 127                         | 48                        | 19                      | 2 10           |                                     |
| 6                             | "          | 75                            | 110                         | 35                        | 15                      | 2 10           |                                     |
| 7                             | "          | 98                            | 152                         | 54                        | ..                      | ..             |                                     |
| 9                             | "          | 90                            | 140                         | 50                        | 12                      | 2 10           |                                     |
| 12                            | "          | 80                            | 131                         | 51                        | 9                       | 2 10           |                                     |
| 15                            | "          | 90                            | 140                         | 50                        | ..                      | ..             | Suckled mother for 10 days.         |
| 17                            | "          | 79                            | 125                         | 46                        | ..                      | ..             | Slight scouring, 2nd and 3rd weeks. |
| 20                            | "          | 90                            | 140                         | 50                        | 2                       | 2 10           |                                     |
| 23                            | "          | 86                            | 145                         | 59                        | ..                      | ..             |                                     |
| 25                            | "          | 88                            | 148                         | 60                        | ..                      | ..             |                                     |
| Total 25 : 13 bull, 12 heifer |            |                               |                             |                           |                         |                |                                     |
| A. M.                         | Bull       | 90.31                         | 147.15                      | 56.85                     | ..                      | ..             |                                     |
| S. D.                         | "          | 11.29                         | 9.59                        | 10.64                     | ..                      | ..             |                                     |
| A. M.                         | Heifer     | 85.08                         | 135.42                      | 50.33                     | ..                      | ..             |                                     |
| S. D.                         | "          | 6.85                          | 11.88                       | 6.37                      | ..                      | ..             |                                     |
| A. M.                         | Both sexes | 87.80                         | 141.52                      | 53.72                     | ..                      | ..             |                                     |
| S. D.                         | "          | 9.78                          | 14.03                       | 7.01                      | ..                      | ..             |                                     |

A. M. = arithmetic mean ; S. D. = standard deviation.

Note.—All animals that were not sold were added to the Sanatorium herd.

The fact that no such difference occurred suggests that pasteurized milk is at least equal in value to raw milk in promoting the growth of young calves.

It is interesting to note that the highest individual gain—80 lb.—in a bull calf and the highest individual gain—63 lb.—in a heifer calf both occurred in animals fed on pasteurized milk (P<sub>7</sub> and P<sub>21</sub>).

It will be observed that in the pasteurized group the average increase in weight of the male animals, instead of being greater than that of the female, was slightly less. This circumstance was due mainly to the occurrence of scouring in the bull calves P<sub>2</sub> and P<sub>5</sub> which retarded their growth during the first three weeks and consequently led to a depression in the average weight of the male animals. In this connection attention has already been drawn, when describing the milk, to the trouble that was experienced from scouring during the early stages of the experiment. This appeared to be due, in some measure at least, to inadequate cooling of the milk after pasteurization leading to gross multiplication of the organisms in the milk destined for the evening feed. It seems probable that this

TABLE II  
*Pasteurized milk group*

| No.                           | Sex    | Weight in lb. at commencement | Weight in lb. after 8 weeks | Increase in weight in lb. | Age in months when sold | Price obtained £. s. | Remarks                           |
|-------------------------------|--------|-------------------------------|-----------------------------|---------------------------|-------------------------|----------------------|-----------------------------------|
| 2                             | Bull   | 77                            | 106                         | 29                        | 2                       | 1 2                  | Scouring for first 3 weeks.       |
| 3                             | "      | 90                            | 140                         | 50                        | 2                       | 1 15                 |                                   |
| 5                             | "      | 90                            | 138                         | 48                        | 2                       | 1 10                 | Scouring for first 2 weeks.       |
| 7                             | "      | 98                            | 178                         | 80                        | 2                       | 1 15                 |                                   |
| 11                            | "      | 95                            | 109                         | 65                        | 2                       | 1 10                 |                                   |
| 17                            | "      | 84                            | 127                         | 43                        | 2                       | —                    | Registered for breeding purposes. |
| 19                            | "      | 73                            | 128                         | 55                        | 2                       | 1 0                  |                                   |
| 22                            | "      | 98                            | 154                         | 56                        | 2                       | 1 10                 |                                   |
| 4                             | Heifer | 94                            | 148                         | 54                        | 2                       | —                    |                                   |
| 6                             | "      | 86                            | 132                         | 46                        | 2                       | —                    |                                   |
| 8                             | "      | 74                            | 126                         | 52                        | 13                      | 9 10                 |                                   |
| 9                             | "      | 84                            | 135                         | 51                        | 2                       | —                    |                                   |
| 10                            | "      | 80                            | 132                         | 52                        | 2                       | —                    |                                   |
| 12                            | "      | 97                            | 150                         | 53                        | 2                       | —                    | Slight scouring during 3rd week.  |
| 13                            | "      | 90                            | 138                         | 48                        | 2                       | —                    |                                   |
| 14                            | "      | 70                            | 118                         | 48                        | 7                       | 3 10                 |                                   |
| 15                            | "      | 82                            | 141                         | 59                        | 2                       | —                    |                                   |
| 16                            | "      | 79                            | 135                         | 56                        | 6                       | 4 10                 |                                   |
| 18                            | "      | 83                            | 141                         | 58                        | 2                       | —                    | Slight scouring during 2nd week.  |
| 21                            | "      | 88                            | 151                         | 63                        | 2                       | —                    |                                   |
| 23                            | "      | 97                            | 158                         | 61                        | 2                       | —                    | Slight scouring during 2nd week.  |
| 24                            | "      | 87                            | 150                         | 63                        | 2                       | —                    |                                   |
| 25                            | "      | 72                            | 121                         | 49                        | 2                       | —                    |                                   |
| Total 23 : 8 bull, 15 heifer. |        |                               |                             |                           |                         |                      |                                   |

TABLE II—(contd.)

| No.   | Sex.       | Weight<br>in lb. at<br>commencement. | Weight<br>in lb.<br>after<br>8 weeks. | Increase<br>in<br>weight<br>in lb. | Age in<br>months<br>when<br>sold. | Price<br>obtained. | Remarks. |
|-------|------------|--------------------------------------|---------------------------------------|------------------------------------|-----------------------------------|--------------------|----------|
| A. M. | Bull       | 88·13                                | 141·39                                | 53·26                              | ..                                | ..                 |          |
| S. D. | ..         | 8·79                                 | 20·09                                 | 14·12                              | ..                                | ..                 |          |
| A. M. | Heifer     | 84·20                                | 138·40                                | 54·20                              | ..                                | ..                 |          |
| S. D. | ..         | 8·12                                 | 11·24                                 | 5·38                               | ..                                | ..                 |          |
| A. M. | Both sexes | 85·57                                | 139·43                                | 53·86                              | ..                                | ..                 |          |
| S. D. | ..         | 8·57                                 | 15·42                                 | 9·43                               | ..                                | ..                 |          |

A. M. = arithmetic mean; S. D. = standard deviation.

Note 1.—Calves P<sub>1</sub> and P<sub>2</sub>, which, for causes already stated in the text, died during the first three weeks of life, have been omitted from this table.

Note 2.—All animals that were not sold were added to the Sanatorium herd.

alone was sufficient to give rise to gastro-intestinal disturbance in animals receiving the pasteurized milk. It is true that in both the raw and the pasteurized groups slight scouring occurred in occasional animals at a later date, but never with the serious results observed in calves P<sub>1</sub> and P<sub>2</sub>.

Table III shows the average weekly increase in weight of the two groups.

TABLE III

*Average weekly increase in weight of the animals in the raw and pasteurized groups*

| Week  | Increase in weight in lb. |             |
|-------|---------------------------|-------------|
|       | Raw                       | Pasteurized |
| 1     | 3·88                      | 3·73        |
| 2     | 4·16                      | 3·92        |
| 3     | 4·40                      | 4·08        |
| 4     | 5·32                      | 5·18        |
| 5     | 7·32                      | 8·17        |
| 6     | 9·16                      | 8·83        |
| 7     | 9·12                      | 9·82        |
| 8     | 10·36                     | 10·13       |
| Total | 53·72                     | 53·86       |

As is to be expected with relatively small numbers of animals, the differences are subject to a certain amount of variation. On the whole the mean increase in weight tended to be slightly higher in the raw than in the pasteurized group during the first month. This was probably due in the main to the depression of the average by one or two animals in the pasteurized group which, as already noted, suffered from scouring during the first two or three weeks of life. In spite of this, the average total increase for the whole experiment was slightly higher for the pasteurized than for the raw animals, the percentage figures being 62.94 and 61.18 respectively.

Compared with calves fed on a normal diet, the gains in weight were undoubtedly low. The amount of milk given, while permitting good skeletal development, was insufficient to fatten the animals. Their weight and their market value at the end of eight weeks were, therefore, inferior to those of calves receiving a more generous diet. As soon as their period of experimental observation was over, and the animals that were not to be sold at once were put on to a liberal mixed diet, their weight increased rapidly and within about two to three months equalled that of normally fed animals.

Throughout the experiment the general condition of the calves was good. Though lean, they appeared perfectly healthy and showed no sign of rickets, or of symptoms referable to anaemia such as have been noted by several workers who have fed calves on an exclusive diet of raw milk [see Cannon, 1931; Knoop *et al.*, 1935]. The slight scouring that occurred in occasional animals after the faulty cooling of the pasteurized milk had been put right, and which was seen in both raw and pasteurized groups, may well have been due to the system of pail-feeding, since it is known that this is liable to give rise to digestive disturbances [Sheehy, 1934]. At no time during the course of the experiment was any lay or professional observer able to distinguish between the two groups of animals.

Towards the close of the experiment heifer R<sub>1</sub>, which was two years old, was served twice at an interval of three weeks, and heifer P<sub>4</sub>, which was twenty-two months old, was served once. Both animals are now 'in calf'.

#### DISCUSSION

The results, which have been set out in detail, without any reservations, need little discussion. They speak for themselves. That slight variation should occur in the behaviour of different animals in the two groups was inevitable, but there is nothing whatever to suggest that they are attributable to the difference in the nature of the milk received, or that the differences as a whole are significant. In some respects the results are surprising. Each one of us at the commencement of the experiment was not only prepared to find a difference, but actually expected to find a difference, in favour of the animals in the raw group. Moreover, the farm hands who looked after the animals were strongly prejudiced against the

use of pasteurized milk for the feeding of the calves. This prejudice, which affected all of us, in favour of raw milk for calf feeding, was based of course on pure *a priori* reasoning without any experimental justification. The outcome of the present experiment seems to leave little doubt that, judged by all the criteria we have used, milk produced in a clean manner from healthy animals and submitted to low-temperature pasteurization followed by adequate cooling is equal in nutritive value to milk from the same source consumed in the raw condition. It may be noted that Blackwood *et al.* [1936], who made a careful study of the assimilation and retention of nitrogen, phosphorus, and calcium by calves, were likewise unable to detect any significant difference between raw and low-temperature pasteurized whole milk.

Reference should perhaps be made to the work of M'Candlish and Black [1935], which has frequently been quoted as showing that raw milk is of higher nutritive value for calves than pasteurized. Such an interpretation seems to us to be unjustified. It is true that in certain small groups the animals fed on raw milk increased in weight more rapidly than those on pasteurized, but in other groups the reverse was the case. If an analysis is made of the figures relating to all the animals, it is found that the weighted mean increase was actually greater in the pasteurized than in the raw group, the percentages being 311.3 and 308.4 respectively.

It is not proposed to enter here into the possible application of this knowledge to practical farming conditions. Suffice it to point out that adequate pasteurization of milk should prove of considerable value in the rearing of healthy calves in diseased herds. Some success has already been reported from California in the eradication of tuberculosis from dairy herds by this method [see Roadhouse and Perry, 1930]. In deciding whether or not to adopt pasteurization, farmers need no longer be deterred by the fear that calves brought up on pasteurized milk will develop less satisfactorily than those brought up on raw, because of any hypothetical nutritive inferiority of the pasteurized product.

#### SUMMARY AND CONCLUSIONS

1. An experiment on the feeding of calves is described, which lasted over two years, and which was carried out on a healthy Shorthorn herd free from tuberculosis and contagious abortion.
2. Alternate animals as they were born were allocated, without any selection whatever, to one or other of two groups. One of these was fed on raw, the other on pasteurized milk. Every animal received its mother's colostrum for three and a half days before being put on the milk diet.
3. The milk used was taken from the mixed morning's milk of the whole herd. It was divided into two parts, one of which was given raw, the other of

which was submitted to low-temperature pasteurization at 145°F. (62.8°C.) for 30 minutes and subsequently cooled. The animals were fed morning and evening on measured quantities that were in strict relationship to their body weight.

4. In addition to the milk, hay was allowed *ad lib.* Observations showed that practically none was eaten during the first month, after which each animal consumed about 1 lb. a day, rising to 3 lb. by the eighth week.

5. With the exception of two weaklings which died after fifteen and twenty-three days respectively from causes apparently unconnected with the nature of their diet, all the animals—twenty-five in the raw and twenty-three in the pasteurized group—thrived well and showed no obvious signs of rickets or anaemia.

6. The average increase in weight over the eight-week period for the animals in the raw group was 53.72 lb., and in the pasteurized group 53.86 lb., or 61.18 and 62.94 per cent respectively. This practical identity in weight increase is all the more surprising in view of the fact that the number of bull calves was very much less in the pasteurized than in the raw group.

7. The highest individual gain among the bull calves—one of 80 lb.—and the highest individual gain among the heifer calves—one of 63 lb.—both occurred in animals fed on pasteurized milk.

8. At no time throughout the experiment was any observer, lay or professional, able to distinguish between the two groups of animals.

9. The diet given, though permitting of good skeletal development, was insufficient to fatten the animals. After they had been transferred, however, to a normal diet at the conclusion of their eight weeks in the experiment, they soon put on weight and within two or three months were indistinguishable in size or condition from animals that had received a more generous diet from birth.

10. There is nothing in these results to suggest that the nutritive value of pasteurized milk for calves is in any way inferior to that of raw milk.

#### ACKNOWLEDGMENT

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## ABSTRACTS

**Control of parasites in sheep.** J. H. RIETZ (*J. Amer. Vet. Med. Assn.*, vol. XC, N. S. vol. 43, No. 2, pp. 163-170, 1937).

THE paper was presented at the seventy-third annual meeting of the American Veterinary Medical Association and in the discussion which followed a number of scientists took part.

The control of parasites is one of the major problems confronting sheep husbandmen throughout the sheep raising areas. The problem is specially acute in enclosed pastures, for the hazards in the life-cycle of the parasite are considerably greater in open-range pastures. The problem of educating the sheep husbandmen in the better methods of control that are devised must be accomplished before widespread control methods can be adopted.

The author carried out experiments at the West Virginia Station on the treatment, proper feeding and management of sheep. In all, he encountered 16 species of parasites consisting of stomach, nodular, hook and whipworms, tapeworms, lungworms, coccidia and grubs. A total of 162 sheep exclusive of the untreated controls, were used in the series of experiments and were handled in lots of 20 to 30 individuals. The different treatments were administered at intervals of 21 days. Faecal examinations were regularly made before, during and after the treatments.

On the basis of the results obtained the writer concluded that 1 to 12 per cent solution of calcium polysulphite, when given in doses varying from 1 to 12 ounces after starving the animals for 24 hours, was irritating to the mucous membranes; was a poor anthelmintic, caused the death of most of the sheep. Tetra chloroethylene, when administered in 5 e.c. doses to animals prepared by starving for 24 hours, was found to reduce nematode egg counts but not so rapidly as was the case with some other preparations. It did not relieve cestode infestation. Three ounces of 1·5 per cent solution of nicotine sulphate (40 per cent), when given after starving for 24 hours, nauseated most of the sheep but was found to be quite effective in the reduction of nematode ova and cestode infestation. Colloidal iodine (Merck) proved ineffective in the removal of nematode and cestode infestations. After starving the animals for 24 hours, an average of 8·15 and 14 treatments with 3 and 4·5 ounces of one per cent solution of copper sulphate resulted in 71·7 and 50·8 per cent reduction in the nematode ova respectively. The cestodes were not affected. On an average of 7·5 treatments, without any previous fasting, with 4·5 ounces of 1 per cent solution of copper sulphate effected a reduction of 81·1 per cent in nematode ova; the cestodes remaining unaffected. An average of 14·8 to 7·1 treatments with 3 ounces of 1·5 per cent solution of copper sulphate resulted in a reduction of 76 to 96·6 per cent in nematode ova. In the former case the animals were not starved previously and the cestode infestations were controlled only partially, while in the latter case the animals were starved for 24 hours prior to treatment and the cestode infestations were completely controlled. In one of the lots receiving the latter treatment the reduction of nematode ova was 98·7 per cent.

A mixture of equal parts of 1·5 per cent solutions of copper sulphate and nicotine sulphate (40 per cent) when administered in doses of 3 ounces resulted in a reduction of 98·2 per cent in nematode ova and a complete control of cestode infestation. An average of seven treatments were given.

The untreated control animals in all the lots showed an increase in parasitic infestations. Mass feeding of copper sulphate in a salt mixture in the ratios of 1 : 30 or 1 : 50 proved not only ineffective but at times dangerous. Finally the author concludes that a 1·5 per cent solution of copper sulphate in dosages of 1 ounce or more for lambs and 3 ounces for adults at intervals of three weeks throughout the year, will effectively control the gastro-intestinal parasites. Fasting for 24 hours before treatment is essential.

The author reports having obtained fair results in the treatment of lungworms with chloroform. He, however, points out that the method is not unattended with danger of losses from pneumonia following anaesthesia. About 250 head of sheep suffering from lungworms were treated with an oily solution of pyrethrum, manufactured from 3 pounds of pyrethrum flowers extracted in one gallon of oil. The solution was injected directly into the trachea in doses of 3 to 6 c.c. Only one treatment was required for 70 to 75 per cent of the sheep treated and only 3 to 5 per cent required a third treatment before the larvae disappeared from the faeces. No losses from pneumonia resulted following the use of this solution.

Good feeding and management are markedly reflected in the control of parasites of sheep. [H. D. S.]

**Immunisation Active des Bovidés contre la Fièvre Aphteuse au moyen d'adsorbats d'aluminium (Active immunization of bovines against foot-and-mouth disease by means of aluminium adsorbates).** M. E. TOUSSIEN (Bulletin de l'Office International des Épidémiologies, xii, 417-444, 1936).

The article records the results of experiments conducted with a view to test if immunity is conferred upon calves that have been vaccinated against foot-and-mouth disease with virus adsorbed on to aluminium hydroxide.

One or the other of the following three strains of viruses was used in the making of the several brews of the experimental vaccine: (a) "Riens O", an "O" strain obtained from Prof. Waldmann, (b) "Hansen-Fionic", a strain recovered from a spontaneous outbreak of foot-and-mouth disease in the Fionic Island and ascertained to be an "O" strain, and (c) "Virus 2", a second "O" strain obtained from Prof. Waldmann.

Some of the brews of vaccine were made each to comprise two portions, the second portion made to contain double the concentration of virus as that contained in the first portion.

Calves that were intended for the test of the efficacy of a vaccine were vaccinated twice at ten days' interval with the brew concerned, first with the portion containing the lower concentration of virus and secondly with that containing the higher. Brews made in single strength were used in much larger quantities for the second inoculation.

The vaccine was diluted considerably with physiological salt solution before use, and injected subcutaneously, divided between various sites. Samples of sera were taken from the calves after the vaccination and titrated on guinea pigs against foot-and-mouth virus with a view to ascertain the degree of immunity acquired (but this did not give clear cut positive results in most instances, except that generalisation was delayed in some vaccinated cases). After an interval of about three weeks after the second vaccination, the calves were tested for immunity by being placed in contact with an artificially infected calf along with a healthy calf for control. If the healthy control calf contracted foot-and-mouth disease and the vaccinated calf remained unattacked, this was taken as evidence of acquired immunity. This was confirmed, in each such case, by submitting the vaccinated calf after an interval of two to three weeks to a further "tongue-test" by inoculation of virus directly into the mucosa of the tongue and upper jaw. As the "Riems O" and "Virus 2" strains proved to be of insufficient virulence for calves, it was necessary to repeat the immunity tests of calves vaccinated with these strains, against the "Hansen-Fionie" strain which was virulent for bovines.

Three types of material were used as the base for the production of the vaccine (i) culture fluid, (ii) extract of aplithous membranes, and (iii) lymph.

(i) *Culture fluid vaccine*.—Culture was effected according to Trenkel's technique. The ingredients were used in the proportion of five parts of virus, five parts of tissue, twenty parts of bovine serum and about two parts of phosphate buffer solution. The tissue and virus were first mixed and allowed to remain in the refrigerator for at least one hour. The other ingredients were then added and incubated for four days. The tissue was then removed and emulsified with quartz sand and phosphate buffer solution. This was centrifuged and the supernatent fluid brought, by further addition of phosphate buffer solution, to the same volume as the culture fluid. This was then filtered alone or mixed with the culture fluid through an EK Seitz filter. The filtrate was stored in the refrigerator. Sometimes filtration was not resorted to, but the supernatent fluid was collected after ultra-centrifugation.

For making the vaccine, 40 parts of the culture fluid was mixed with 60 parts of aluminium hydroxide. One brew, the fourth, was made in two concentrations, the one intended for the first vaccination containing 10 per cent culture fluid and the other intended for the second, containing 20 per cent of the culture fluid. Four brews were made, the first and second from "Riems O", the third from "Hansen-Fionie" and the fourth from "Virus 2". Five calves were vaccinated. Four of these contracted foot-and-mouth disease during the contact test. The fifth one (a "Riems O") withstood contact test, but was susceptible to the "tongue-test".

(ii) *Tissue extract vaccine*.—Aplithous membrane was obtained from one or more infected animals, and crushed with quartz sand. After ultra-centrifugation (at 15,000 for 15 minutes), the extract was diluted in physiological saline solution to increase it to about ten times the weight of the tissue. Each brew of vaccine was made in two concentrations—the one intended for the first vaccination containing 20 parts of the extract and 80 parts of aluminium hydroxide, and the other intended for the second vaccination containing 40 parts of the extract and 60 parts of aluminium hydroxide.

Three brews of tissue extract vaccine were made. The first and second brews were made from aphthous membranes of calves infected with the "Hansen-Fionie" and the third from aphthous membranes of swine infected with "Virus 2". The first vaccine in the third brew was a formalised one.

Six calves were vaccinated with the tissue extract vaccine. Two calves vaccinated with the second brew contracted generalised foot-and-mouth after the first vaccination on account of incomplete adsorption of the virus and were of no use for purposes of the experiment. One calf in the first brew had become solidly immune. Of three calves in the third brew two were susceptible by contact and the third developed a suspicious vesicle but without pyrexia. The latter was found to be immune to the subsequent "tongue-test".

(iii) *Lymph vaccine*.—Only one brew of lymph vaccine was made. Lymph was collected from swine that had been inoculated into the snout, coronets and clefts of hoofs with "Virus 2". The lymph was initially diluted ten times. The first vaccine contained ten parts of 1/10 diluted lymph and ninety parts of aluminium hydroxide and the second contained twenty parts of the diluted lymph and eighty parts of aluminium hydroxide. Three calves were vaccinated with this vaccine. Two of them were found to be solidly immune. The third was resistant to contact infection, but contracted foot-and-mouth disease (without generalisation) when submitted later to the "tongue-test".

To sum up the results of the experiment, fourteen calves were vaccinated twice at ten days' interval with aluminium hydroxide adsorbates of foot-and-mouth virus. Two of these contracted foot-and-mouth following the first vaccination. This was due to incomplete adsorption. The remaining twelve calves were exposed about three weeks after the second vaccination, to infection by contact, which caused generalised foot-and-mouth in six. The other six remained insusceptible to contact infection.

The latter six calves were inoculated, two to three weeks later, directly into the mucous membrane of the tongue and jaw. While this inoculation had always induced generalised foot-and-mouth in the control animals, only two of the six vaccinated calves became attacked, and only one of them had shown generalisation.

[The results recorded in this article, although promising, cannot be regarded as conclusive on account of the small number of animals involved, and inconsistency of results in some groups. In addition some animals were submitted to more than one immunity test, with the result that any immunity detected at the final test could not be safely ascribed as due solely to the vaccination.] [V. R. R.]

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**Inheritance of resistance of mice to enteric bacterial and neurotropic virus infections.** WEBSTER, L. T. (*J. Exp. Med.* 65, 2, 261-286, 1937).

Previous studies by the same author had shown that certain factors regulating the resistance of mice to naturally induced *Bacillus enteritidis* infection were inborn. In this article he has presented new data to show that, under experimental conditions resistance to *B. enteritidis* and resistance to St. Louis encephalitis virus are inherited in a similar and relatively definite manner but independently.

Of all the existing lines, susceptible and resistant to natural *B. enteritidis* infection, only susceptible lines 1 and 2, which showed a relatively stable mortality rate of approximately 85 per cent from the outset and resistant lines 1 and 2, which showed a fluctuating mortality rate of about 15 to 20 per cent were retained for further selective breeding and the rest were discarded. Unselected controls showed a relatively stable mortality rate of 37 per cent. Selections for breeding were made as previously, from litters unexposed to infection, thereby insuring against introduction or persistence of the infection in the stock. The method has been to remove the first and often the second litter from the breeding room at 4 to 6 weeks of age and test each with *B. enteritidis* or virus. If the mortality was maximum or minimum as required, an additional sibling litter was selected and mated brother to sister without testing. First and second litters of the succeeding generations were then tested in the same manner and a third litter chosen for mating. The mouse colony was subjected to continued search for infectious agents. The occasional sickly mouse was sacrificed and tested for the presence of pathogenic bacteria or virus. Faeces from breeders were tested 6 to 12 times a year for mouse typhoid.

Selections of mice for breeding had been made entirely on basis of maximum susceptibility or resistance of progeny to *B. enteritidis*. The susceptible and resistant lines which developed were later found to be susceptible and resistant respectively to *Pasteurella avicida*, *B. friedlander* and pneumococci administered intranasally. Accordingly the former has been designated as bacteria-susceptible (BS) lines and the latter as bacteria-resistant (BR). But, in 1932 and 1933, when it was shown that BS lines were resistant to louping ill virus and BR lines susceptible, it became necessary to complicate the terminology further and to identify the one as bacteria-susceptible-virus-resistant (BSVR) lines and the other as bacteria-resistant-virus-susceptible (BRVS) lines. Finding the mortalities of BSVR lines to louping ill virus averaging about 50 per cent and often concentrated only in certain litters led the author to attempt to develop sub-lines highly resistant to virus (DSVR) and highly susceptible to virus (BSVS) respectively. Parents whose progeny had shown least and greatest mortality following nasal instillation of louping ill virus were selected for further breeding; all others in these bacteria-susceptible lines were discarded. Subsequent tests were made with St. Louis encephalitis virus.

Rigid selection, testing and discarding procedures on approximately 13,200 mice for twelve generations from 1930 to November 1934, resulted in three inbred lines which have shown percentage mortalities to *B. enteritidis* and virus averaging as follows: BSVS, 95.2 and 86.3 per cent; BSVR, 96.2 and 6.7 per cent; BRVS, 17.3 and 96.6 per cent respectively. High susceptibility proved relatively stable throughout, while high resistance appeared to fluctuate from generation to generation. The susceptible lines showed high mortalities following doses as little as 1/100th of the standard and the resistant lines showed low mortalities following doses 100 times the standard. Consequently, the resistant lines, both bacterial and virus, were considered to withstand 1,000 to 10,000 times the dose fatal for susceptibles.

The results of the cross-breeding tests support the selective breeding data in indicating that resistance to these infections is controlled basically by inherited factors and confirm the previous findings that:

- (1) the factors are not sex-linked,
- (2) resistance is dominant over susceptibility.

- (3) the factors regulating resistance to *B. enteritidis* are not related to those regulating resistance to encephalitis virus, and
- (4) the mechanism of this inheritance may be relatively simple, since the mortality percentages in the cross-breed generations approximate for the most part those expected on the basis of two single factor crossings.

When BSVR strain was crossed with BRVS, bacteria-resistant-virus-resistant (BRVR) mice were encountered. Hence it was assumed that among these cross-breds, a pure BRVR strain might be present which could be segregated from heterozygous BRVR reactors by selective breeding. Certain lines have thus emerged from this which to date are resistant to both *B. enteritidis* and virus.

All selected lines proved uniformly susceptible to a strain of mouse passage rabies virus, given by natural or artificial routes. [R. L. K.]

#### The problem of grass silage :—

1. The chemical composition of grass silage. S. J. WATSON and W. S. FERGUSON. (*J. Agric. Sci.* 27, 1-42, 1937).
  2. Losses of dry matter and digestible nutrients in low-temperature silage with and without added molasses or mineral acids. S. J. WATSON and W. S. FERGUSON. (*J. Agric. Sci.* 27, 67-107, 1937).
  3. Grass Silage. A comparison of the changes involved in the ordinary molasses and A. I. V. Processes. W. MORLEY DAVIES, G. H. BATHAM and W. B. THOMPSON. (*J. Agric. Sci.* 27, 151-161, 1937).
1. THE main chemical changes which take place during different processes of ensilage are studied in this paper. The study extends to 293 samples of silage made in towers, wood-lined pits and stacks or clamps.

Detailed methods of analysis are given for pH, crude protein, volatile base, amino-acid, lactic acid, total volatile acids, acetic acid and butyric acid contents. These determinations afford a means of assessing the nutritive value of silage.

*By ordinary process.*—46 per cent of the silages show a pH value of 4.5—5.0, 31 per cent a pH value of 4.0—4.5, and only 4 samples had a value below 4.0. The greater formation of volatile bases and volatile acids and an appreciable butyric fermentation make this process decidedly inferior to the A. I. V. Process. High protein material fares worse but with fully mature material, a good compaction of material and maximum exclusion of air give satisfactory results.

*By addition of molasses.*—An addition of about 1—2 per cent of molasses suitably diluted gives very good silages. 47 per cent of the samples show a pH value of 4.0—4.5 and 29 per cent under 4.0. But the breakdown of protein, the formation of volatile bases, the quantity of amino-acids and the more complex nitrogenous compounds place this product only next in quality to the product formed by the A. I. V. Process, although the butyric fermentation is controlled and the lactic fermentation promoted.

*By addition of whey.*—These samples show an average pH value of 3.86 and give a product comparable with that made by the addition of molasses.

*By addition of moderate amounts of mineral acids with or without molasses.*—The average pH of this product is 4·32 and the silage had no apparent advantage over the molasses process.

*By the A. I. V. Process.*—This results in a silage of excellent quality in which the breakdown of protein, the formation of organic acids including butyric, and the production of volatile bases are reduced to a minimum.

2. This paper dealing with experiments extending over a period of five years is an attempt to assess the losses involved in the various processes of ensilage from a practical point of view.

Practical details are given of the process of making the different types of silage. The technique adopted for correctly estimating the losses of dry matter is discussed and it is concluded that only figures obtained from silos where 8 to 10 tons are filled would apply under field conditions.

*Loss of Dry matter.*—There was no appreciable difference in dry matter losses between the various types of silage.

*Loss of Starch equivalent.*—The ordinary silage shows the largest losses, all the others having about 5–10 per cent less. But these values, when corrected for the products of protein breakdown, show less differences between the different types. The A. I. V. Silage and that made by the addition of whey show the lowest losses being 21·7 and 17·6 respectively.

*Loss of Protein equivalent.*—The A. I. V. Product and the whey product have the minimum losses of this constituent being 21 per cent and 21·1 per cent respectively. The molasses silage shows a high loss and the ordinary product the highest loss. It has, however, been shown that the breakdown products of true protein are of as high a nutritive value as the true protein itself, if the process has not proceeded beyond the amino-acid stage. If this fact is conceded the differences between the various types disappear.

*Loss of Digestible crude protein.*—The losses of digestible crude protein are very low and agree well with the losses of protein equivalent. There is no appreciable difference between the various types.

These results are discussed in relation to those of other workers.

It is concluded that although the A. I. V. Process gives the lowest losses, the molasses process is not significantly deficient in meeting practical requirements. With material of advanced maturity the ordinary process of ensilage is quite satisfactory.

3. The process of making ordinary, molasses and A. I. V. Silages are briefly described. The technique employed in sampling and analysis is also given.

With the exception of an increase in the ether extract, all other fractions showed losses on ensilage.

*Ordinary Process.*—This resulted in a dry matter loss of 25·1 per cent crude and 32·5 per cent digestible and a loss of 36·2 per cent starch equivalent. The digestibility of this silage is 60·3 per cent.

*Molasses Process.*—This gives a loss of dry matter of 12·5 per cent crude and 17·1 per cent of digestible and a loss of starch equivalent of 24·0 per cent. The digestibility of this silage is 64·3 per cent.

*A. I. V. Process.*—This shows a loss of dry matter of 18·1 per cent crude and 13·3 per cent digestible and a loss of starch equivalent of 12·8 per cent. The digestibility of this silage is 71·8 per cent.

The molasses process is preferred on account of the simplicity of the process and the comparatively good quality of the product obtained. [P. A. S.]

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**Télégonie (Telegony).** LETARD, E. (*Rec. Méd. Vét.* exii, 513-527, 1936).

This article is in the nature of a semi-popular note in which the author tries to combat the popular belief of the stock-breeders in the existence of telegony.

Telegony is defined as the supposed influence of a sire previously mated with a female, on offspring subsequently borne by that female to a different sire. A female is "impregnated" by the first male to which she is bred, with the result that all subsequent offspring, regardless of their actual father, show resemblances to the first male.

The belief is based, not on carefully controlled experiments, but on facts of observation. From a number of these observations which are typical of those advanced in favour of telegony, a few examples, selected at random, are described:

(i) A white swine, served by a wild boar for the first time, brought forth young ones which were brown like the father. This swine was subsequently mated to males of her own race, and always some of the little ones born of these services presented on certain regions the dark pigmentation of the wild boar.

(ii) A black cow of the Angus race, a race characterised by hornlessness, was served for the first time by a horned Durham bull. It was subsequently served by a bull of the Angus race. But of their coupling was born a horned roan-coloured calf.

(iii) A black and hairless bitch was accidentally covered by a cross-bred spaniel with long brown hair. It brought forth five pups three of which were without hair and two covered with brown short hair. Mated later to a Turkish dog, black and hairless like itself, half the pups of this second litter resembled the mother, but the other half resembled those born of the coupling with the first sire.

(iv) A bitch with bald skin and of black colour, served by a brown spaniel, gave birth to five pups three of which were hairless while the other two were covered with short brown hair. Covered subsequently by a black and hairless dog of its own breed, it gave birth to pups, some of which had coats resembling that of the spaniel which covered her for the first time.

These and similar authentic observations, combined with the support given to the theory by naturalists, biologists, and evolutionary philosophers, and publicity given by novelists, historians and jurisprudence have left a strong belief among breeders in the reality of telegony.

In trying to combat this belief, the author proceeds to explain in the first place, that no sustainable scientific explanation has been advanced in support of telegony. Among the various theories that have been advanced in its favour, critical examination is made of one that is mostly in vogue, namely, the saturation hypothesis. According to this hypothesis the foetus has in its blood special properties derived from the father and on account of the existence of exchange between the maternal and foetal blood, the constitution of the mother may be affected with the result that subsequent offsprings by another father may be influenced. But this conception is directly opposed to the well established chromogenic theory of heredity. In conducting experiments in animal genetics with cross-breds, particularly with reference to the transmission of a dominant character, it is found that the sex-cells are not influenced by the bodily peculiarities of the animal which lodges them. No more direct evidence is required to show that the bodily peculiarities of an animal do not circulate in its blood in any form. Such being the case, it is all the more unconceivable that the farther removed embryo could have any influence on the sex-cells of its host.

Besides, experiments carefully planned to investigate the problem of telegony, undertaken by various workers with a view to reconstruct certain remarkable cases that had seemed to demonstrate the existence of telegony, had always failed to yield positive results.

Nevertheless, the fact remains, that certain observations, examples of which have been given above, have appeared to uphold the existence of telegony. The author explains that while these facts are authentic their explanation has been faulty. Their true explanation is to be found in the Mendelian law of heredity.

The Mendelian law of heredity shows that there is not always agreement between the external characters of an individual (phenotype), and those which it can transmit to its descendants (genotype). According to circumstance, there is, or there is not, agreement between the phenotype and the genotype. A parent with a long line of ancestors selected for the same attributes shall transmit all these attributes with constancy when it is served with the same stock as itself. In this case the virtues or shortcomings displayed truly correspond to the qualities which it will transmit to its descendants: here there is agreement between the phenotype and the genotype. On the contrary, let one take an individual born out of the crossing of two individuals of different races. It is possible that it remarkably resembles one of its parents. Nevertheless one knows that not only it can, but must, certainly transmit to its descendants such attributes that it did not externally display, but which have been held in latency in the sexual cells. In this case there is disagreement between the phenotype and the genotype. It is thus possible sometimes, that bodily peculiarities of an ancestor may sometimes make their appearance in an offspring removed by several generations.

The author quotes as an example the case of the crossing of a grey mouse of pure breed with a white mouse. Greyness being the dominant colour, the young ones will be grey like the grey parent. These grey cross-bred descendants, served between themselves, will always give a fourth of white mice. This phenomenon is constant. A cross-bred grey mouse, if covered by a white male, will give according to the Mendelian law, half of grey ones and half of white ones. If this same cross-bred grey mouse were served afterwards with a grey cross-bred male, she will give as aforesaid, a fourth of

white descendants. One who did not know the cross-bred origin of these mice may put this down as due to the influence of the first father. But the same result would have been realised even if the latter mating had taken place first. It is unavoidable. To utilise telegony in order to explain this is, therefore, radically wrong.

The author explains that the cases he quoted earlier as examples of those advanced in favour of telegony are again nothing but instances of operation of the ordinary Mendelian law of heredity.

With reference to the case of two white swine giving birth to pigmented piglings, the explanation lies in the fact that matings between swine and wild boar are not rare, and that a wild boar should have been used in the past in building up this race of swine. A recent experience of L'école d'Alfort is illustrative. Some years ago matings were made between the wild boar and the Large-White swine of the school. Through selection, and elimination of the tainted, a strain was built having all the appearance of a pure race of Large-White. Two of these perfectly white subjects have now given birth to a piglet with wild boar characteristics.

With reference to the black Angus bovines without horns giving rise to a roan subject with horns, the author advances a similar explanation. The two Angus parents should have had a horned roan coloured ancestor in the distant past. One knows that the absence of horns and black colour dominate over the presence of horns and roan coloration. It is, therefore, possible that the latter characters have remained latent in some animals as recessive factors. When two such animals accidentally come together, certain of their offsprings get these factors in the duplex condition with the result that the recessive factors become exteriorised.

The author explains the case of two furless dogs giving rise to a furred one, as only an expression of the existence of a dominant "lethal character". Hairless skin in the dog is a lethal character, that is to say, young ones that could have this character in the duplex condition and be genotypically hairless are never born, but suffer death in their embryonic life. The hairless dogs are therefore all cross-breds, and will never breed true but always throw a proportion of furred offspring. This happens invariably without exception. The lethal character is a proven factor in genetics, and the case in question is easily explained thus without having to invoke telegony.

It is therefore concluded that telegony is a myth. [V. R. R.]

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**Report of the Special Committee appointed by the National Veterinary Medical Association to study the subject of Small Animal Euthanasia, 1937. (Published by the National Veterinary Medical Association of Great Britain and Ireland, London. Price 5s., 1937.)**

At the instance of the National Veterinary Medical Association of Great Britain and Ireland, a special Committee was appointed to study and express opinion on the existing methods of painless destruction of small animals. "The remit to the Committee was in the following terms:—

- "(a) That the Committee be requested to explore in all its bearings the subject of small-animal euthanasia, i.e. of dogs, cats and birds with a view to arriving at an authoritative opinion as to which is the most efficacious method of humanely destroying these animals.

"(b) That the Committee be asked to express their opinion upon the methods at present in use—in addition to the other methods which the Committee might feel disposed to test—with particular regard to the question whether one or more of them can be described as 'cruel' or 'painful':—

- |   |   |
|---|---|
| 1. HYDROCYANIC ACID . . . . .                                       | Oral, intrathoracic, intracardiac, intravenous.   |
| 2. CHLOROFORM . . . . .   | Inhalation, nasal injection, intrathoracic, intracardiac, intravenous. Pre-narcotisation with Morphine, Chloral Hydrate, Chloretone, Nembutal, Avertin. |
| 3. MAGNESIUM SULPHATE,<br>STRYCHNINE.                               | Intravenous and intracardiac injection.   |
| 4. LETHAL CHAMBERS . . . . .  | Chloroform, Coal Gas, Carbon Monoxide, motor-car exhaust fumes.   |
| 5. SHOOTING . . . . .   | Including captive bolt pistols.   |
| 6. ELECTRIC SHOCK   |   |
| 7. ANY METHOD that might be recommended for destruction 'en masse.' |   |

"(c) That it should be left to the Committee to decide if their observations should be applied to apparently healthy animals and/or to aged, diseased or unhealthy animals."

The laborious work, entailed in covering such wide terms of reference, was carried out simultaneously yet independently at five centres where there is a Veterinary College and extensive tests were carried out with large number of cats, dogs and often birds.

The subject matter in the text of the report is dealt with lucidly under the following headings:—

- (1) Agents in common use.
- (2) Pre-narcotization by the use of non-volatile anaesthetics and narcotics.
- (3) Chloroform as lethal agent.
- (4) Lethal chambers.
- (5) Destruction by shooting.
- (6) Euthanasia by electric means.
- (7) The mass destruction of dogs and cats, and
- (8) Commentary and conclusions.

Investigations into the use of strychnine was not included in the programme as the members of the Committee were firmly convinced in regard to its painful and distressful character.

Hydrocyanic acid without pre-narcotisation was not recommended because of the resultant distress and pain that preceded death. The certainty of its action, however, prompted the investigation on suitable narcotics whose prior administration might reduce or even eliminate altogether the objectionable features of this product. As a result morphine and chloral hydrate were definitely rejected. Nembutal (Abbot) though costly for large dogs, yielded the most consistent and satisfactory results. Intrathoracic, intraperitoneal and intravenous routes have been recommended but the last method

was found by the Committee to be the most satisfactory for dogs and cats of all sizes. The recurrent tarsal vein in the dog and radial vein in the cat should be used and the dose should not be less than  $\frac{1}{2}$  of a grain per pound body weight (0.045 grm. per kilo) for small animals and not less than  $\frac{1}{2}$  grain per pound body weight (0.034 grm. per kilo) for large animals." Intravenous administration of Evipan sodium (Bayer) is also recommended. The dose employed should not be less than  $\frac{1}{2}$  grain per pound body weight (0.09 grm. per kilo) in 10 per cent solution. In the dog, the intravenous use of "Fluid Avertin" (Bayer) at the rate of 1 c. c. per 15 lb. body weight is also considered a humane and satisfactory method of euthanasia. This agent is not, however, recommended for general adoption in cats. Chloroform, which is widely used as a lethal agent, was administered by various routes and it was observed that in general it was not desirable for cats but was valuable for dogs if given intramuscularly or by inhalation after proper morphine narcosis.

It is not generally recognised that magnesium sulphate when introduced directly into blood stream is a powerful and rapidly acting narcotic poison. The intravenous use of this agent is in the opinion of the Committee a very satisfactory and reliable method of painless destruction of dogs. It was observed that accidental introduction of the solution in subcutaneous tissues did not produce any painful result and in such an eventuality it is recommended that injection in some other vein or other method of euthanasia should be adopted. The technique is fully described and the concentration of the solution recommended is 100 grains of the hydrated salt in 100 ml. of water injected at 40 to 50° C. The dose advocated is from 5 ml. in the case of small terriers to 20 ml. in large dogs such as Airedale terriers.

The efficacy of the lethal chambers depending on the use of chloroform in combination with carbon dioxide, motor-car exhaust fumes and coal gas, was extensively investigated but is not recommended as it was observed that their use was not free from distress.

The action and use of captive-bolt pistols is discussed in detail and the Committee observed that this method of destruction is most rapid and humane. When the premises are suitable it is the method of choice for dogs. No assistance is required, the degree of skill necessary is small and accidents are rare. This method is not considered suitable for cats due to gross contamination of the fur by blood consequent to the involuntary movement which occurs prior to cessation of heart beats.

Two methods of electric shock employed for the humane destruction of small animals, namely electric stunning and the cabinet method, were dealt with *in extenso* as also the working and construction of the requisite appliances. Both are considered rapid and humane provided the technique, which does not require great skill or assistance, is correct. The chief cost is the initial outlay. Where large numbers of unwanted animals are to be destroyed, the use of an electric chamber is considered to be by far the best and is strongly recommended.

That this valuable report, emanating from the labours of such an eminent personnel of the Committee, supplies a real need there can be no question. It should prove an acquisition to all interested in the subject. [H.B.S.]

**Raw vs. Pasteurised Milk.** DR. C. CROWTHER. *Journal of the Ministry of Agriculture*, 14, 4, July 1937, p. 391).

EXPERIMENTS in feeding rats on a diet of milk and white flour at the National Institute for Research in Dairying, have shown that both raw and pasteurised milks could sustain life whereas sterilized milk could not do so. No difference could be detected between raw milk and commercially pasteurised milk by the holding method, either in the digestibility and biological value of the protein or in the availability of the calcium and phosphorus and the former did not prove inferior to the latter when used as an exclusive diet supplemented by iron, copper and manganese. The conclusions arrived at by the Milk Production Department of the West of Scotland Agricultural College, after feeding trials with 35 dairy calves, are of much importance in calf rearing. The calves were divided into different groups of winter heifers and winter bulls to be tried for 150 days and summer heifers and summer bulls to be tried for 120 days. Colostrum of the dam was fed to 4 groups for 5 days and to the rest for 10 days after which raw or milk pasteurised by heating at 145-150°F for 30 minutes were fed at the rate of 1 lb. per 10 lb. live-weight until a maximum daily allowance of 15 lb. was reached. After the third week the calves had free access to good hay and an allowance of concentrates. The winter bull calves on raw milk and those on pasteurised milk after 5 days colostrum feeding showed an increase of 364 and 330 per cent respectively of their live-weight at birth in 150 days. The spring bull calves fed on raw milk and pasteurised milk after 5 days of colostrum feeding showed an increase of 272 and 244 per cent respectively of their live-weight at birth in 120 days. The heifer calves fed on pasteurised milk after 10 days of colostrum feeding and those after 5 days of colostrum feeding, took 2 months and 4 months respectively to overtake the heifer calves fed on raw milk and at the end of the first year the heifers on raw milk had made a live-weight gain of 500 per cent while those on pasteurised milk after 10 days colostrum had gained 554 per cent. Thus very small superiority of raw milk over pasteurised milk is observed. But the calves on raw milk were observed to have better coats and incidence of diseases also seemed to be less for the group, it being the heavier the shorter the initial period on colostrum. More extensive experiments on a larger number of calves are necessary before definite conclusions are arrived at. [H. L.]



## NOTES

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### CHANGES IN TITLES OF SOME OF THE IMPERIAL AGRICULTURAL BUREAUX

In pursuance of the recommendations of the British Commonwealth Scientific Conference 1936, the Executive Council of the Imperial Agricultural Bureaux have decided to adopt the following revised titles for certain bureaux with effect from the first January 1938 :—

- (a) The Imperial Bureau of Plant Breeding and Genetics (at Cambridge).
  - (b) The Imperial Bureau of Pastures and Forage Crops (at Aberystwyth).
  - (c) The Imperial Bureau of Horticulture and Plantation Crops (at East Malling).
  - (d) The Imperial Bureau of Animal Breeding and Genetics (at Edinburgh).
  - (e) The Imperial Bureau of Agricultural Parasitology (Helminthology) (at St. Albans).
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### THE THIRD INTERNATIONAL CONGRESS FOR MICROBIOLOGY

THE Third International Congress for Microbiology will be held at the Waldorf-Astoria Hotel, New York City, September 2-9, 1939, under the auspices of the International Association of Microbiologists.

T. M. RIVERS, M. D., President, Rockefeller Institute for Medical Research, York Avenue and 66th Street, New York City.

M. H. DAWSON, M. D., General Secretary, College of Physicians and Surgeons, 620, West 168th Street, New York City.

KENNETH GOODNER, Ph.D., General Treasurer, Rockefeller Institute for Medical Research, York Avenue and 66th Street, New York City.

The Congress will be composed of the following nine sections :

1. General Biology : Variation and Taxonomy. Convener : C. E. A. WINSLOW.
2. General Biology : Microbiological Chemistry, and Physiology. Convener STUART MUDD.

## NOTES

3. Viruses and Viral Diseases. Convener : W. A. SAWYER.
4. Rickettsiae and Rickettsial Diseases. Convener : HANS ZINSSER.
5. Protozoology and parasitology. Convener : H. W. STUNKARD.
6. Fungi and Fungous Diseases. Convener : B. O. DODGE.
7. Medical and Veterinary Bacteriology. Convener : F. P. GAY.
8. Agricultural and Industrial Microbiology. Convener : S. A. WAKSMAN.
9. Immunology. Convener : M. HEIDELBERGER.

Registration fee will be \$5.00, which will not include the cost of a banquet ticket or a copy of the Proceedings of the Congress.

A World's Fair will be held in New York City during the summer of 1939. Consequently, those who wish to attend the Congress for Microbiology should make plans promptly. The American Express Co., the Official Travel Agency for the Congress, will be glad to assist in such plans.

# PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

[Prices are inclusive of packing and Indian Postage]

## 1. Agriculture and Live-stock in India

- ICAR. 1 A bi-monthly journal of agriculture and animal husbandry for the general reader interested in agriculture or live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9s. 9d. per annum. Price per part Rs. 2 or 3s. 6d.) Volumes I to VII complete are available.

## 2. The Indian Journal of Agricultural Science

- ICAR. 5 A bi-monthly scientific journal of agriculture and the allied sciences mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24s. per annum. Price per part Rs. 3 or 5s. 3d.) Volumes I to VII complete are available.

## 3. The Indian Journal of Veterinary Science and Animal Husbandry

- ICAR. 6 A quarterly journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931. Published in March, June, September and December. Prepayable subscription Rs. 6 or 9s. 9d. Price per part Rs. 2 or 3s. 6d.) Volumes I to VII complete are available.

## 4. Scientific Monographs of the Imperial Council of Agricultural Research

- ICAR. 10-1 No. 1. The Fungi of India, By E. J. Butler, C.I.E., D.Sc., M.B., F.R.S., and G. R. Bishy, Ph. D. (1931). Price Rs. 6-12-0 or 11s.
- ICAR. 10-2 No. 2. Life-histories of Indian Microlepidoptera, Second Series : Alucitidae (Pterophoridae, Tortricina and Gelechiidae), By T. Bainbridge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 3-4-0 or 5s. 6d.
- ICAR. 10-3 No. 3. The Open Pan System of White-Sugar Manufacture, By R. C. Srivastava, B.Sc. (1935, 2nd edition.) Price Rs. 3-2-0 or 5s. 6d.
- ICAR. 10-4 No. 4. Life-histories of Indian Microlepidoptera : Cosmopterygidae to Neopseustidae, By T. Bainbridge Fletcher, R.N., F.L.S., F.E.S., F.Z.S (1933). Price Rs. 4-8-0 or 7s. 6d.
- ICAR. 10-5 No. 5. The Bombay Grasses, By E. J. Blatter, S. J., Ph. D., F.L.S. and C. McCann, F.L.S. Illustrated by R. K. Bhide. (1935). Price Rs. 20-12-0 or 32s. 6d.
- ICAR. 10-6 No. 6. Helminth Parasites of the Domesticated Animals in India, By G. D. Bhalero, M.Sc. (1935). Price Rs. 7-12-0 or 13s. 3d.

## I. C. A. R. PUBLICATIONS

- ICAR. 10-7 No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease, By W. McRae, M.A., D.Sc. (Edin.), F.I.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.I.S. (1933). Price Rs. 2-4-0 or 4s. 3d.
- ICAR. 10-8 No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, By C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6s. 9d.
- ICAR. 10-9 No. 9. Mechanical Cultivation in India. A History of the Large-scale Experiments carried out by Burmah-Shell Oil Storage and Distributing Company of India, Ltd., By C. P. G. Wade. (1935). Price Rs. 3-14-0 or 6s. 6d.
- ICAR. 10-10 No. 10. The Spotted Boll-Worms of Cotton in South Gujarat, By B. P. Deshpande and N. T. Nadkarni, B.Ag. (1936). Price Rs. 5-14-0 or 9s. 6d.
- ICAR. 10-11 No. 11. Investigations on the Course and Distribution of the Nerves supplying Levator anguli scapuli and Rhomboideus muscles and the formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations, By H. N. Chelva Ayyangar, G.M.V.C. (1937). Price Rs. 4-10-0 or 7s. 9d.
- ICAR. 10-12 No. 12. The Fungi of India. Supplement I. By B. P. Mundkur. (*In the Press*.)

## 5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

- ICAR. 8-1 No. 1. List of Publications on Indian Entomology, 1930, By the Imperial Entomologist, Pusa. (1934). Price As. 14 or 1s. 6d.
- ICAR. 8-2 No. 2. List of Publications on Indian Entomology, 1931, By the Imperial Entomologist, Pusa. (1934). Price As. 8 or 10d.
- ICAR. 8-3 No. 3. List of Publications on Indian Entomology, 1932, By the Imperial Entomologist, Pusa. (1934). Price As. 12 or 1s. 3d.
- ICAR. 8-4 No. 4. Host Plant Index of Indo-Ceylonese Coccidae, By S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. (1934) Price Rs. 1-10-0 or 2s. 9d.
- ICAR. 8-5 No. 5. List of Publications on Indian Entomology, 1933, compiled by the Imperial Entomologist, Pusa. (1935). Price As. 9 or 1s.
- ICAR. 8-6 No. 6. Bee-keeping, By C. C. Ghosh, B.A., F.E.S. (3rd Edition.) (1936). Price Rs. 1-14-0 or 3s. 3d.
- ICAR. 8-7 No. 7. List of Publications on Indian Entomology, 1934, compiled by the Imperial Entomologist, Pusa. (1935). Price Rs. 1-2-0 or 2s.
- ICAR. 8-8 No. 8. Selected Clinical Articles, Part I, By G. K. Sharma, G.P.V.C. and R. L. Kaura, B.V.Sc., M.R.C.V.S. (1936). Price As. 8 or 10d.
- ICAR. 8-9 No. 9. Statistical Methods and their Application to Agronomy : A bibliography, By K. K. Guha Roy. (1936). Price Rs. 2-2-0 or 4s.
- ICAR. 8-10 No. 10. Diseases of Sugarcane and Methods for their Control, By L. S. Subramaniam. (1936). Price Re. 1 As. 14 or 3s. 3d.
- ICAR. 8-11 No. 11. Tables of Standard Errors of Mendelian Ratios, compiled by Swami Singh Purewal, M.Sc., Ph.D. (Cornell) and P. Krishna Rao, L. Ag. (1936). Price As. 12 or 1s. 3d.

- ICAR. 8-12 No. 12. List of Publications on the Botany of Indian Crops, Part II, for the period 1928-32, By R. D. Bose. (1936). Price Rs. 3-6-0 or 6s. 9d.
- ICAR. 8-13 No. 13. Two New Statistical Tables based upon Fisher's 't', By M. Vaidyanathan. (1936). Price As. 6 or 8d.
- ICAR. 8-14 No. 14. List of Publications on Indian Entomology (1935), compiled by the Imperial Entomologist, Pusa. (1937). Price Re. 1-4-0 or 2s.
- ICAR. 8-15 No. 15. Selected Clinical Articles, Part II, By G. K. Sharma, G.P.V.C., R. L. Kaura, B.V.Sc., M.R.C.V.S., S. Ganapathy Iyer, G.M.V.C., G. S. Khan, B.Sc. and S. Mangrulkar, M.Sc., M.R.C.V.S., D.T.V.M. (Eds.) (1937). Price Re. 1-4 or 2s.
- ICAR. 8-16 Indian Grazing Conditions and the Mineral Contents of some Indian Feeders by P. E. Lander, M.A., D.Sc., F.I.C., I.A.S. (1937). Price Rs. 3-14 or 6s. 9d.
- ICAR. 8-17 A Brief Survey of some of the Important Breeds of Cattle in India by Col. Sir Arthur Oliver, C.B., C.M.G., F.R.C.V.S., F.N.I. (1938). Price Rs. 2 or 3s. 6d.
- ICAR. 8-18 Milk Records of Cattle in Approved Dairy Farms in India by K. P. R. Kartha. (*In the Press.*)
- ICAR. 8-19 No. 19. A Preliminary Annotated List of the Fruit Pests of the North-West Frontier Province by Hem Singh Pruthi M.Sc., Ph.D. (Cantab.), F.N.I. and H. N. Bhatia, B.Sc., Assoc. I. A. R. L. (*In the Press.*)

#### 6. Annual Report of the Imperial Council of Agricultural Research

- ICAR. 12-31 Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1s. 3d.
- ICAR. 12-32 Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8d.
- ICAR. 12-33 Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8d. (Out of Stock.)
- ICAR. 12-34 Annual Report of the Imperial Council of Agricultural Research for the year 1933-34. Price Re. 1-8-0 or 2s. 6d.
- ICAR. 12-35 Annual Report of the Imperial Council of Agricultural Research for the year 1934-35. Price Re. 1 or 1s. 9d.
- ICAR. 12-36 Annual Report of the Imperial Council of Agricultural Research for the year 1935-36. Price As. 14 or 1s. 6d.
- ICAR. 12-37 Annual Report of the Imperial Council of Agricultural Research for the year 1936-37. Price Re. 1-2-0 or 2s.

#### 7. Agriculture and Animal Husbandry in India (called Review of Agricultural Operations in India up to 1933)

- ICAR. 9-29 Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2-0 or 5s. 6d. (Out of stock.)
- ICAR. 9-31 Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8s. 3d.
- ICAR. 9-33 Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9s. 6d.
- ICAR. 13-1-35 Agriculture and Animal Husbandry in India, 1933-34 and 1934-35—  
Part I.—Crop Production. Price Re. 4-14-0 or 8s.
- ICAR. 13-2-35 Part II.—Animal Husbandry. Price Re. 1 or 1s. 9d.
- ICAR. 13-36 Agriculture and Animal Husbandry in India, 1935-36. Price Rs. 4-10 or 7s. 9d.

**8. Proceedings of the Board of Agriculture and Animal Husbandry.**

- ICAR. 2 Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9s. 6d.
- ICAR. 3 Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices. Price Rs. 6 or 9s. 9d.

**9. Miscellaneous Publications.**

- VRI. 1 A Description of the Imperial Institute of Veterinary Research, Muktawar, and its Sub-station, the Imperial Veterinary Serum Institute, Izatnagar, By F. Ware, F.R.C.V.S. Price Rs. 1-4-0 or 2s.
- ARLT. 187 The Production of Cigarette Tobacco by Flue-curing, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Insp. Inst. Agri. Res., Proc. Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1s. 9d.
- ICAR. 16 A Handbook of Statistics for use in Plant Breeding and Agricultural Problems, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7s. 3d.
- ICAR. 7 Report on the Work of the Imperial Council of Agricultural Research in Applying Sciences to Crop Production in India, By Sir John Russell, D.Sc., F.R.S. Price Rs. 1-14 or 3s. 3d.
- ICAR. 18 Report on the Development of the Cattle and Dairy Industries of India, By Norman C. Wright, M.A., D.Sc., Ph. D. Price Rs. 1-8-0 or 2s. 6d.

Copies of the above publications can be had from :—

The Manager of Publications, Civil Lines, Delhi and from any of the Agents, a list of whom will be found on the inside page of the front cover.

Purchasers not residing in Asia, Africa and Australia should apply to the High Commissioner for India, India House, Aldwych, London. Prices include Indian postage and packing.

*Note.*—When indenting please give only the symbol preceding the name of the publication.

## APPENDIX

### Instructions to Authors of Publications of the Imperial Council of Agricultural Research\*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuations should be done in the MS. and not left for introduction in proofs.
2. The title of a paper should not be lengthy.
3. It is desirable that MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper Roman numerals should be used. The use of Arabic figures and (a), (b), (c), etc., is generally reserved for numbering the sub-divisions coming under each head.
4. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandry* should be accompanied by abstracts for publication in *Agriculture and Live-stock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with; ordinarily no abstract should exceed 200 words.
5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in **SM. CAP.** with two lines, in **CAPITALS** with three lines, and when in **Antique** (heavy type) with a wavy line (~~~~~).
6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.
7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, e.g., *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, e.g., *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, e.g., *Striped Mauritius*, *Dharwar-American cotton* and *Broad cotton*.
8. Botanical and zoological names are printed in italics and should be underlined in the MS., e.g., *Triticum vulgare L.*; *Diplodia Corchori Syd.*; *Pyrilla aberrans*

\*Spare copies of these Instructions can be had on application to the Secretary Imperial Council of Agricultural Research (Publication Section), New Delhi.

Kirby. The International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter; they are printed in roman type (e.g., calcium carbonate, prussic acid).

9. The following and similar abbreviations may be used freely—viz., e.g., i.e., mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundred-weight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattack). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word "see" or "cf.", in the MS. itself, and should not be left over for introduction in proofs. For example, "The parasite (Plate X, fig. 4) was present late in 1906".

11. The word "Table" is preferable to "Statement", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally; for this purpose they should not exceed in width the printing measure of the page (5 in.). Example—

TABLE IV

*Results of water-saving experiments on wheat (*Pusa 12*) at Gungapur, Haripur and Sargodha, 1916-17*

| Station        | No. of irrigations including the preliminary watering | Yield per acre in maunds and seers |        | Average yield per acre |           |
|----------------|---|------------------------------------|--------|------------------------|-----------|
|                |   | Grain                              | Straw  | Grain                  | Straw     |
| Gungapur . . . | One   | 12 19½                             | 20 10  | Mds. Srs.              | Mds. Srs. |
| Haripur . . .  | "   | 8 31                               | 19 14  | 9 34                   | 21 17     |
| Sargodha . . . | "   | 8 12½                              | 25 27½ |                        |           |

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be

indicated by the author's name followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after the year both in the text and in the collected references. This system of referencing is the same as is used in the *Biochemical Journal* with slight modification and will be clear from the following illustration:—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

#### REFERENCES

- Coward, K. H. and Drummond, J. C. (1921). *Biochem. J.* **15**, 530.  
Osborne, T. B. and Mendel, L. B. (1919, 1). *J. Biol. Chem.* **37**, 187.  
\_\_\_\_\_. (1919, 2). *J. Biol. Chem.* **41**, 549.  
Steenbock, H. and Boutwell, R. (1919). *J. Biol. Chem.* **41**, 149.  
Wilson, J. (1922). *J. Biol. Chem.* **51**, 455.

Abbreviations, as far as possible, should follow the system adopted in "A World List of Scientific Periodicals" published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the "Standard Dictionary" should be used. Second (page) proofs will be submitted to authors who should return them promptly.

#### Illustrations

14. As the format of the journals has been standardized, the size adopted being crown quarto (about  $7\frac{1}{2}$  in.  $\times$   $9\frac{1}{2}$  in. cut), no text-figure, when printed, should exceed  $4\frac{1}{2} \times 5$  inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being  $5\frac{1}{2} \times 8$  inches exclusive of that for letterpress printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are understood; thus, "half-size" means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should

always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil-painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on "Photographic illustrations" in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on "The Preparation and Reproduction of Scientific Illustrations" in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa*.

GIPD—765 IC of AR—19-3-38—650.

## ORIGINAL ARTICLES

### THE INTER-RELATIVITY OF THE DISEASES OF ANIMALS AND MAN\*

BY

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Editor of the Veterinary Journal.*

(Received for publication on 2nd February, 1938)

IN India as well as in England there are certain diseases of animals and man which are of mutual interest to the medical man and the veterinarian, either on account of their analogies or their differences; or by reason of the fact that they are contagious from animals to man or vice versa, and it is not wise for either branch of medicine, nor yet for our mutual patients, to work in watertight compartments. It is better from every point of view that we work in collaboration. Some diseases, such as cancer, tuberculosis, anthrax, and tetanus we attack respectively in somewhat different ways; whilst others, such as glanders, rabies, foot-and-mouth disease, mange, and ringworm can only be effectively dealt with by definite collaboration between medical men and veterinarians. There are others in which as yet there has been no effectual attempt at collaboration. Such ailments as common catarrh and the influenzas can be dealt with to mutual advantage from a comparative aspect, as can more complicated ailments such as Hodgkin's disease, and such common ailments as rheumatism and fibrosis. An exchange of ideas as to the symptoms, methods of spread, etc., in our various patients is of undoubtedly help towards elucidation. In veterinary medicine, equally with the human side, the study of collateral branches of science, such as entomology or parasitology, is of material help, and in teaching colleges, as in hospitals, a knowledge of the life-histories of the various flies and insects which act as carriers or transmitters of parasites is as essential to the veterinary student as to his medical *confrère*. In the clinical world, too, we have many points in common, for our animal patients suffer from gastritis, indigestion, colic, internal parasites, colitis, swallowing of foreign bodies, and various forms of pneumonia and heart disease, to the same extent as human patients do, and our veterinary treatments are similar in principle to those in human practice. The veterinarian has, however, a greater variety of internal arrangements to deal with, having to take into account whether his patient is

\* Paper presented to the Indian Science Congress, Calcutta, January, 1938.

herbivorous or carnivorous; or whether, as is the case in man, it will eat anything and everything which it has an opportunity of eating. Some of our patients have only one stomach, whereas others have four—while the camel stands by itself in having three—so that their respective digestive processes vary very much in detail.

I feel sure that when a number of these diseases, both epidemic and otherwise, are studied from this point of view, we shall be able to advance more quickly and find many new ideas and theories, which up to the present have not been thought of. It is not only in Great Britain that diseases may be studied in this way, for those who live in the tropics have also plenty of opportunity for following up comparative medicine. The different effects which various foods have on man and animals also form a good illustration. For example, the flour of certain forms of Indian pea has a nerve-paralysing effect not only on the natives continually fed on it but also on horses, producing laryngeal paralysis which causes dyspnoea on the slightest exertion. Again in entomology in the study of the life-histories of the various flies and insects which act as carriers and transmitters of disease-germs or blood-parasites, the knowledge acquired by collaboration is of mutual benefit in epidemiology, not only in the diseases transmitted from animal to animal but in those transmitted from animal to man. In the short time now at my disposal I shall confine myself to a selection of a few diseases concerned with Public Health, which are communicable from animals to man, in the treatment of which the practitioner of human medicine can obtain material help from collaboration with his veterinary *confrère*.

#### GLANDERS

This is primarily a disease of the horse tribe, and affects horses, asses, and mules. Its cause—the *Bacillus mallei*—is an extremely dangerous organism to work with in the laboratory. The disease is one which is most commonly met with amongst stable-workers and those who come in contact with horses, and a man can be readily infected by the discharge from the nostrils of an infected horse, or even by handling the brushes, sponges, or stable-cloths, which have been in contact with a glandered horse. In the South African war it accounted for the deaths of many thousands of our Army horses, and indeed in all wars it has been the bugbear for which the army veterinary officer must always be on the look-out.

It is so insidious that, until it has been present in the system for a certain length of time, its presence may remain unsuspected. Modern veterinary science has now, however, at its command a method by which the presence of glanders can be ascertained, for by the introduction of a few drops of mallein (a special preparation made from the *Bacillus mallei* itself) the skilled veterinarian can make a diagnosis with certainty within forty-eight hours, even if the animal is infected only in the slightest degree. During the Great War, by means of this test, applied by the officers of the Royal Army Veterinary Corps, glanders was entirely eradicated from the horses and mules

of the British Army, and it has been applied so successfully in Great Britain that at the present time the disease has absolutely ceased to exist. This means that not only has it been eliminated from the list of ailments which the veterinary surgeon is called upon to diagnose, but it has also been eliminated from the list of diseases in man; and in an island country like Great Britain, so long as the present regulations of the Veterinary Department of the Ministry of Agriculture and Fisheries are kept in operation, the country will be free from this terrible affection. In India I understand that it is still a problem to be dealt with, but of this I hope to hear something in the discussion.

#### RABIES

This disease has not been met with in man in England for more than thirty years, and it can never appear again as an epidemic in this country so long as control is kept upon the importation of animals of the dog and cat tribe. The primary cause of rabies in man is the contact of an abraded surface of the body with the saliva of a rabid animal, and whether the infected animal is a horse, a sheep, or any other animal, it has always had its primary origin in a rabid dog and cat. The Muzzling Order succeeded in eradicating the disease from Great Britain, and it then remained for the Veterinary Advisers to the Ministry of Agriculture and Fisheries to take steps to see that it was not re-introduced into the country. This explains the present quarantine regulations imposed on all dogs and cats admitted from countries where rabies exists. The absence of the disease is further proof of the value of the collaboration between the forces of the veterinarian and the medical man in the cause of Public Health. In India you are much more heavily handicapped than we are in England, especially on account of the numbers of pariah dogs, over which, I understand, it is difficult to obtain control.

#### ANTHRAX

This condition is much more prevalent in India than in England, and is particularly met with in cattle, horses, sheep and pigs; the dog, cat and fowl possessing a comparatively high power of resistance to the infection. It is a disease which is always serious and, in animals, invariably results in death. In cattle, especially, death is very sudden, and in Great Britain the Government has imposed laws and regulations which provide that the body must be cremated as near as possible to the place where the animal died. It is forbidden, too, in any way to cut the carcase, for on many occasions these making, or assisting at, the *post mortem* have become infected and have died in consequence. In cities and towns in England where wool from foreign countries is handled, disinfection is compulsorily adopted, with satisfactory results. If this practice could also be efficiently adopted in the case of hides, bone manure and other animal products, before they are imported into this country, the number of deaths from anthrax in man and animal would diminish considerably. Cotton, linseed, and other cattle-food cakes come into the same

category. Once eradicate anthrax from the animal and animal products and eradication from man would automatically follow. Anthrax is primarily a disease for the Veterinary Surgeon, as it always originates from some products obtained—or used—by an animal.

#### FOOT-AND-MOUTH DISEASE

This disease has at times, in our English Press, provoked a good deal of unwarranted criticism directed against the Veterinary Advisers to the Ministry of Agriculture and Fisheries, yet there is no doubt that, in England, they have adhered to the correct policy (that of "Stamping out"). We have much upon which to congratulate ourselves when we compare our position with that of other European countries. The cost to Holland, France, Belgium, Denmark and Germany, amounts to tremendous sums each year, and these countries never get any further forward in the matter—having the disease always endemic. The fact that we are an island is of incalculable value to our Ministry of Agriculture, whose responsibility it is to frame the laws which control the importation of animals from any country from which infection may be brought.

The following statistical table, showing the respective numbers of outbreaks in other European countries during 1934, is convincing evidence of the value of the "Stamping out" method in an island country like Great Britain :—

| Month               | Great Britain | France | Germany | Holland | Belgium |
|---------------------|---------------|--------|---------|---------|---------|
| January . . . . .   | 1             | 1,074  | 113     | 579     | 329     |
| February . . . . .  | ..            | 652    | 80      | 214     | 168     |
| March . . . . .     | ..            | 613    | 73      | 105     | 102     |
| April . . . . .     | ..            | 287    | 110     | 59      | 81      |
| May . . . . .       | 1             | 135    | 48      | 51      | 40      |
| June . . . . .      | ..            | 146    | 56      | 132     | 36      |
| July . . . . .      | ..            | 98     | 27      | 459     | 19      |
| August . . . . .    | 3             | 92     | 40      | 1,391   | 15      |
| September . . . . . | 4             | 21     | 19      | 3,120   | 20      |
| October . . . . .   | 24            | 15     | 14      | 2,880   | 9       |
| November . . . . .  | 28            | 3      | 16      | 48      | 1,173   |
| December . . . . .  | 18            | 28     | 32      | 230     | 20      |

The public should think what a terrible disaster it would mean to a small confined country like England if the disease were allowed to spread with the fact before it that milk from cattle affected with foot-and-mouth disease must not on any account be consumed by children or invalids, or be given to goats, pigs, or any other animal.

In India it is always more or less with you but in a much milder form than we get it in Europe, and the conditions do not necessitate the same drastic treatment nor would such be possible.

#### TUBERCULOSIS

This is pre-eminently a disease which illustrates the value of collaboration between the medical man and the veterinarian in the cause of Public Health. No variety of domesticated animal is immune to tuberculosis, although some are more susceptible than others. The goat, the sheep, and the horse, are probably the least affected, but even in these it is only a question of degree and there is no actual immunity when they are placed under conditions favourable for infection. Birds, especially poultry, are frequently affected and whenever the disease appears amongst them the whole flock may have to be destroyed before it is eradicated.

It is a disease which the practising veterinarian meets with most commonly in cattle, and there are about a million tuberculous cattle in Great Britain at the present time. These are not all dairy cattle, but it is in these that the danger lies for man, as it is well known that at least 40 per cent—and, in some districts, 60 per cent—of them are affected.

At one of the National Milk Conferences, Dr. Stanley Griffith, in a paper on "Bovine tuberculosis and its relation to man", gave some statistics which went to prove more than ever the necessity for medical practitioners and veterinarians to pull together. In an investigation of 1,200 cases of tuberculosis he had found that 87·5 per cent of infections with tuberculosis of the cervical glands, in children up to the age of 5, were bovine; and similarly 61·3 per cent of those between 5 and 10 years; 37·9 per cent of those between 10 and 16 years; and 25 per cent of those of 16 years and over. Of 476 cases of bone-and-joint tuberculosis, 28·7 per cent of those under 5 years were of bovine origin; 23·1 per cent of those between 5 and 10 years; 9·5 per cent of those between 10 and 16 years; and 6·4 per cent of those of 16 years and over. Of 126 cases of lupus: 69 per cent of those under 5 years; 42·5 per cent of those between 5 and 10 years; 60 per cent of those between 10 and 16 years; and 17·6 per cent of those of 16 years and over were of bovine origin. The same medical scientist estimates that tuberculosis contracted through the consumption of cow's milk causes approximately 3,000 deaths in young children every year. As all these infections are caused by drinking the milk of cows suffering from tuberculosis of the milk glands, it is hoped that the new regulations of the Live-Stock Industry Bill which comes into active operation in April of this year (1938) will, so far as Great Britain is concerned, have the effect of eliminating the chances of infection from the cow to man in Great Britain.

Although only about one per cent of dairy cattle is affected in the udder, until the infection has reached this organ the milk does not necessarily contain tubercle bacilli. An infected cow is always a possible source of danger,

for one can never tell exactly when the udder tissues will become infected ; and the milk a source of definite and terrible danger to the children to whom it is given.

Pasteurization, undoubtedly, offers some safeguard, but it is generally admitted that some of the valuable properties which raw milk possesses are lost during this process, and there can be no doubt that the best solution of the prevention of infection lies in the endeavour to obtain an absence of the tubercle bacilli at the source of supply—*i.e.*, the dairy herd. That this can be accomplished, if pecuniary and other necessary adjuncts are available, has been proved by actual experiments, and America has been especially go-ahead in her endeavours to form accredited herds. In that country whole districts have been cleared, and the most stringent laws are enforced in order to prevent reinfection by the entrance of tuberculous beasts into these areas.

In Great Britain progress in this direction has been slow ; as the British public, although not unmindful of the advantages of tuberculosis-free milk, is not willing, as a body, to pay an extra price for this guarantee. Dairy-men who have gone to the expense and trouble of clearing their herds have not received the encouragement they deserve either from the general public or from the hospitals and medical practitioners. These last, in particular, might do a very great deal more than they are doing to assist in educating the housewives and mothers of young children as to the dangers of tuberculous milk, by urging upon them the necessity for demanding a clean milk supply, *i.e.*, one from tuberculin-tested cows.

This matter is now being seriously taken in hand, and a Veterinary State Service has been formed, with a staff of whole-time men whose duties consist mainly of the inspection and testing of dairy cattle, with a view to the formation of tuberculosis-free herds. A clinical inspection is made of the udders periodically, usually four times a year, and for the owner who wishes to ensure that his herd is completely free from tuberculosis, the cows are tested with tuberculin—which we now have a synthetic variety—and by the intradermal method which forms a much more delicate test than the former subcutaneous method. We have reason to hope that this newly formed Veterinary State Service is thoroughly justifying its existence and that it will prove of benefit not only to human beings, by getting rid of a source of tuberculous milk, but also to the dairyman and the agriculturist, by weeding out from his herds tuberculous cattle whose presence is always a source of danger. It is a common observation that the herds from which tuberculosis has been eliminated are much more resistant to other ailments—the services of the veterinary surgeon being less in demand than when this disease existed.

#### MANGE

Mange of the horse is now dealt with in all parts of Great Britain and is compulsorily notifiable under a Mange Order issued from the Ministry of Agriculture and Fisheries. Its spread has been effectually checked, and although it is not yet completely eradicated, the number of cases in the horse

is now extraordinarily small. It is, however, to the domestic pets, especially the dog and cat, that attention should be drawn, for it is quite an easy matter for a pet dog to transmit the parasite of mange from itself to its owner. An itchy dog should, therefore, always be regarded with suspicion, and the pernicious habit of allowing a dog to sleep in bed with a human being should be emphatically discouraged. A dog with mange, especially in hot weather, or when its body becomes heated by lying in front of the fire (or sleeping on an eider-down or blanket), will be continually scratching, especially in the region of the arm-pits and under the thighs, where the body is hot and the hair is thin. If no treatment is adopted, the dog will break out in sores, the hair will fall off, and the animal will presently smell very offensively and become covered with scabs. If allowed to come into contact with any part of the human body for more than a few minutes, it is quite an easy matter for the parasite to transfer itself to its human host, and it may remain for a considerable number of days, or even weeks, until it has finished its life-history. During this time it will give rise to a great deal of irritation and discomfort, which could easily have been prevented had the owner of the dog sought veterinary advice.

There are numbers of other diseases in which it is of value to the Public Health Service that, in the fight for their eradication, the human physician and the veterinarian should collaborate, for the patients of each are equally attacked. Cancer may be taken as a type. This dreaded disease is recognised in such veterinary patients as horses, cattle, dogs, cats and even fish, and many of the theories which research workers form, if their observations are concentrated on man alone, may at once be seen to be erroneous upon comparing notes with veterinary pathologists, whose lives bring them in contact with the comparative aspect. In foreign countries this has been for a long time recognised, and their governments have granted liberal funds for research into the problems of animal diseases and their relation to Public Health, finding it a paying proposition—even if considered only from the economic standpoint. Great Britain has been behindhand in this respect, but during the past few years with the establishment of the Animal Research Institute connected with the Royal Veterinary College, at Camden Town, and of the Institute of Animal Pathology at Cambridge, together with the creation of University Veterinary degrees and a Post-graduate Diploma of Veterinary State Medicine, there is a good prospect that, long before another decade has passed, the Government organisation of Veterinary Officers of Health will have as important a place in Public Health as is accorded to the graduates of the human branch of State Medicine.

My list of those diseases which furnish valuable instruction in the epidemiology of animals and man is by no means complete, but I must in conclusion just allude to one other—namely, contagious abortion of cattle which gives rise to Undulant Fever of man. That cases of transmission from the cow do occur is generally admitted, but not as frequently as might be expected when one considers the great prevalence of this disease in milking cattle.

In connection with milk, too, as a food product one must not forget to draw attention to its danger as a carrier of disease in such diseases as scarlet fever and diphtheria of man.

In conclusion, I hope that you will agree that I have introduced sufficient framework in the brief time at my disposal to illustrate the importance of consideration of the important relationship existing between the diseases of animals and man.

**BABESIA BOVIS STARCOVICI, 1893, AS THE CAUSE OF  
RED-WATER IN AN INDIAN BUFFALO\***

BY

J. A. IDNANI, G.B.V.C.

*Imperial Veterinary Research Institute, Mukteswar.*

(Received for publication on 13th December 1937)

(With Plate III)

The purpose of this article is to record, for the first time in India, the occurrence of *Babesia bovis* Starcovici, 1893, in the blood of a buffalo.

Bovine red-water in this country implies the invasion of red cells of the blood with *Babesia bigemina* [Smith and Kilbourne, 1893] which is so widespread that indigenous cattle, being in a state of 'Carrier' immunity, generally bear the effects caused by this parasite with impunity.

*Babesia bigemina*, as it occurs in India, has always been observed to conform with the usual morphological picture ascribed to it in literature from other parts of the world. It is fairly large in size, the dimensions generally conforming with those described originally by Smith and Kilbourne [1893] and subsequently by other systematists [Wenyon, 1926], viz., 2  $\mu$  to 4  $\mu$  in length and 1.5  $\mu$  to 2  $\mu$  in width at the broadest part. It usually occurs in double pear-shaped forms which converge at their pointed ends forming a very acute angle, with the result that the bulbous ends lie fairly closely together. The parasite almost always occupies the centre of the red cell and consists of a cytoplasm easily stained with basic dyes, and of a fairly big chromatin mass staining deeply with acid dyes. It is not quite uncommon for one, specially in those cases where an active multiplication of the parasite is going on, to come across a number of red blood cells invaded by a variety of forms resulting from the amoeboid activities of the parasite. In such cases the form most commonly met with is that where the parasite is observed to be almost circular, the cytoplasm appearing to be condensed towards the periphery with quite a big clear central area. The clinical manifestations (fever, haemoglobinuria) caused by the acute effects of the parasite can usually be relieved by one or two injections of a suitable dose of the dye, trypanblue, which is extensively employed against infection with *B. bigemina*.

Cattle in this country commonly harbour another variety of piroplasm, viz., *Theileria mutans*, which is of an even more widespread occurrence than *B. bigemina*. The morphological features of this parasite, however, are too familiar to give rise to any confusion between these two different families of Piroplasmidea.

\* Paper read at the Indian Science Congress held at Calcutta, January, 1938.

## HISTORY OF THE CASE UNDER REPORT

The parasite which is the subject of the present article was encountered in the blood smears from an indigenous buffalo, Sita, of the Military Dairy Farm, Belgaum, which were forwarded to this Institute on 7th August, 1936, for microscopical examination with the following history:—

The animal was found to be ailing on the 4th August with a temperature of  $104\cdot0^{\circ}$  F. On the following day its temperature rose to  $105\cdot0^{\circ}$  F. in the morning, and to  $105\cdot2^{\circ}$  F. in the evening. The next day (i.e., on 6th August), the animal is said to have passed 'blood' in the urine (haemoglobinuria ?) and to have showed a further rise in temperature to  $105\cdot6^{\circ}$  F. One hundred and twenty cubic centimetres of a one per cent solution of trypanblue were administered intravenously on that day at 11 A.M. At noon the temperature further rose to  $106\cdot2^{\circ}$  F., but dropped to  $103\cdot4^{\circ}$  F. in the evening. Another injection of 100 c.c. of one per cent trypanblue solution was given on the subsequent day in the same manner, as the temperature again rose to  $105\cdot2^{\circ}$  F. It was on this day that blood films were made and sent to this Institute for diagnosis.

## DESCRIPTION OF THE PARASITE AND DIAGNOSIS

The smears, on microscopic examination after staining with Giemsa, presented marked anaemic changes in the shape of anisocytosis, poikilocytosis, and punctate basophilia. About twenty-five per cent of the red cells were found to have been invaded by an unusually small and slender parasite. The parasite was observed, on merits of its morphological features, to fall under the generic head *Babesia*. The criteria which led to its differentiation from *B. bigemina* were as follows:—

(1) *The very small size of the parasite.*—Its longest diameter ranged between  $0\cdot5 \mu$  in the smallest individuals and  $2 \mu$  in the biggest parasites. Similar dimensions for *B. bigemina* as stated above are  $2 \mu$  to  $4 \mu$ .

(2) *The arrangement of the double pears.*—The twin parasites, when they occurred in pairs, were observed to be widely divergent at their blunt extremities, so much so that in some cases they appeared to lie in one straight line. Such an arrangement is extremely rare with *B. bigemina*.

(3) *The position of the parasite in the red cell.*—It was observed that the parasite had a remarkable tendency to lie towards the periphery of the invaded cells. It has already been stated that *B. bigemina*, as a rule occupies the centre of the cell. Further, two injections of trypanblue failed to bring about ameliorations of the infection. All these factors, taken into consideration together, left no doubt about the parasite concerned being a species different from *B. bigemina*, and resembling the parasite (*Babesia bovis*) described by MacFadyean and Stockman [1911] as *Babesia divergens*. The writer's diagnosis was confirmed by Mr. F. Ware, Director, and later by Dr. H. N. Ray, Systematic Protozoologist at this Institute, both of whom possess personal acquaintance with *Babesia bovis* as it occurs in English cattle. It is





*Babesia bovis*,  $\times 1600$

worthy of note that the buffalo in question was housed alongside some European cattle at the Belgaum Dairy Farm.

It may be mentioned that an attempt was made to transmit the disease in buffaloes and hill bulls with the blood obtained from the infected buffalo but this failed. This failure may be attributed to the haemolysed and decomposed state in which the sample of infective blood employed in the transmission experiment was received.

The annexed *camera lucida* drawing (Plate III) represents the morphological characters of most of the *Babesia* parasites as seen in the blood smears from the Belgaum buffalo.

#### SUMMARY

- (1) *Babesia bigemina* has been the only parasite so far known to cause bovine red-water in India.
- (2) The paper records the occurrence in an indigenous buffalo, of another species of *Babesia*.
- (3) The parasite is observed to be different from *Babesia bigemina*, but resembling *Babesia bovis*.

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## DURATION OF IMMUNITY FOLLOWING GOAT-VIRUS VACCINATION IN CATTLE\*

BY

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(With six text-figures)

GOAT-ADAPTED-RINDERPEST-VIRUS was first given a trial on a batch of twenty animals on 17th September 1932, in this presidency, with a view to use it as a prophylactic agent against rinderpest which was responsible in causing, on the average, 11,369 deaths among cattle every year. This experiment proved a success inasmuch as the reaction produced was mild and there were no deaths among the vaccinated animals. Being encouraged by these results, further experiments were undertaken on a larger number of animals with the same results as obtained in the first experiment. It was then considered that this method of preventive inoculations should be given a thorough trial in clean and affected villages and in all available breeds of oxen and buffaloes in different tracts of the presidency. Accordingly, carefully devised experiments were carried out for a period of four years, during the course of which 14,807 animals were subjected to the vaccination operation. The results obtained proved that this vaccination was the cheapest, efficient, safe and suitable method of prophylaxis which is available to afford active immunity in cattle and to cut short rinderpest outbreaks under rural conditions in this country. Basing on these findings, preventive inoculation with anti-rinderpest serum, which was affording protection only for nine days, was totally discarded; a depot for the production of goat-virus was organised in the Bombay Veterinary College and goat-virus vaccination has now been resorted to in the Bombay Presidency, thus making a substantial advance in the method of controlling the fell disease.

The next step in this connection to pursue was to ascertain the duration of immunity conferred by this vaccination. Immunity experiments were, therefore, systematically carried out on vaccinated animals at progressive intervals during previous years with the result that the vaccinated animals were found possessing solid immunity up to four years (Annual Administration Reports of the Veterinary Investigation Officer, Bombay Presidency, Bombay, for the years 1933-34, 1934-35, 1935-36 and 1936-37). Now, with a view to study the subject further a fresh immunity experiment was undertaken and this report deals with the same.

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\* Paper read at the Indian Science Congress, Calcutta, 1938.

## SUBJECTS

Four out of eighty-two animals which were vaccinated with goat-virus on 1st December, 1932, at the Dairy of the Ramabai Mukti Mission (American), Kedgaon, were selected for the test. They were the following :—

Bullock, Paithan, 9½ years.

Buffalo-cow, Gujar, 8½ years, pregnant 2 months.

Cow, Gawali, 10 years, pregnant 8 months.

Buffalo-cow, Shobha, 10 years, milching.

As regards the reaction induced by goat-virus vaccination five years ago in these animals, fever, dullness, congested mucous membrane were common in all ; buffalo-cow, Gujar, had passed very soft faeces but had fed well, all others had impaired appetite and buffalo-cow, Shobha, had shooting diarrhoea. They were in good condition at the time of subjecting to the immunity test.

## VIRULENT RINDERPEST VIRUS

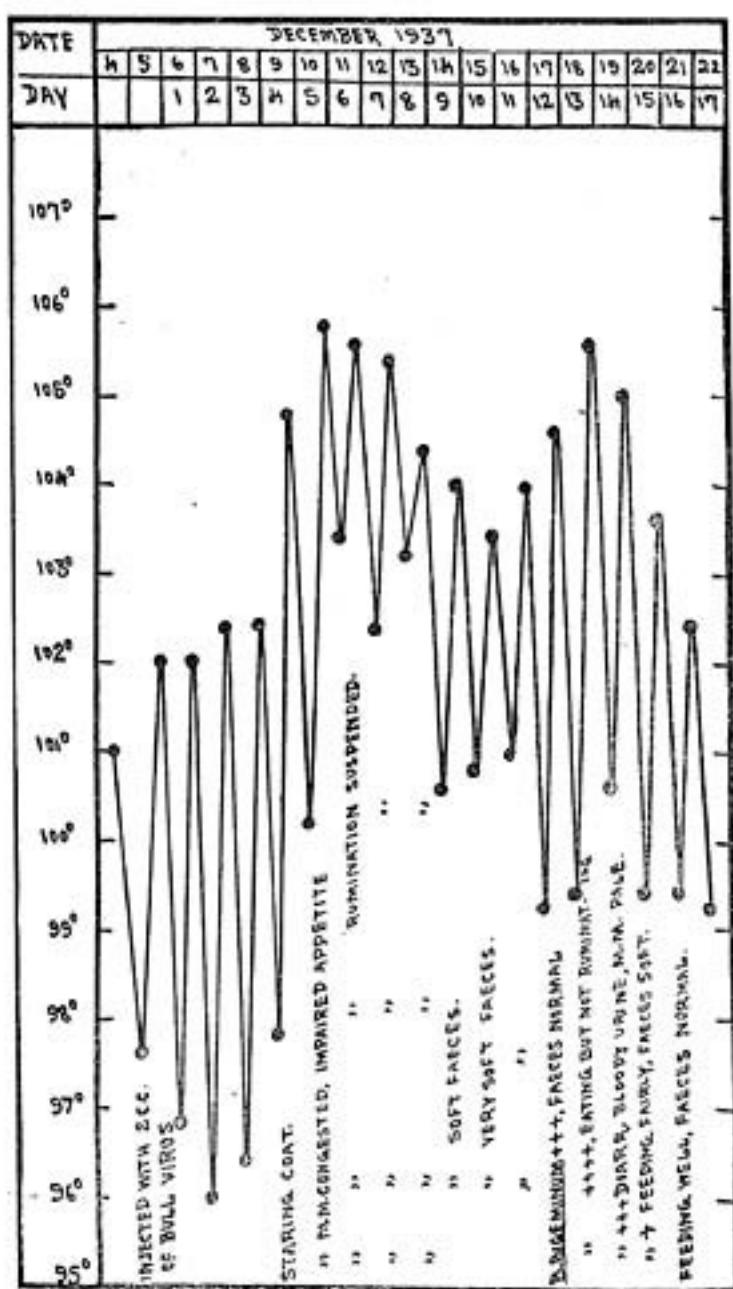
The test was carried out by using virulent rinderpest virus with a view to give a severe trial to the immunity acquired by the animals and for this purpose a consignment of bull-virus from the Imperial Veterinary Research Institute, Mukteswar, was obtained. The virus was collected there on 1st December, 1937, and was received at Kedgaon by post on 5th December, 1937. It was received in an excellent condition of preservation.

## CONTROLS

Two bulls, one two years of age and another three and half years old, which were known to have not suffered from rinderpest previously, were bought at Yevat and transported to Kedgaon, covering a distance of six miles. They were quite healthy and their body condition was fairly good.

## IMMUNITY TEST

The four vaccinated animals and the two controls were injected, each with 2 c.c. of the bull-virus, on 5th December, 1937, and they were housed in a segregation camp specially arranged. They were kept under observation up to 21st December, 1937, i.e., for sixteen days, during which period the reaction produced in individual animals was carefully noted and recorded in the temperature charts (Figs. 1 to 6). A summary of the same is given in Table I.



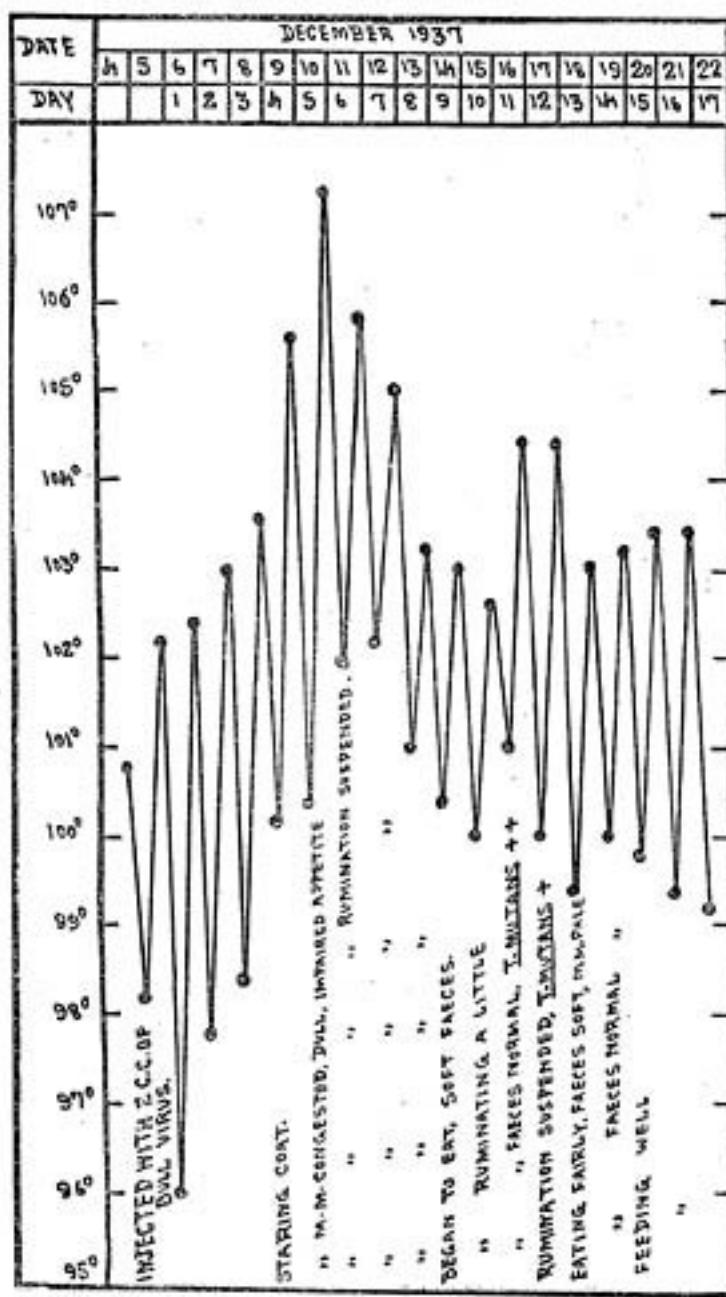
*Goat-Virus Vaccination in Cattle*

Fig. 2.  
Control No. 2. Bull, black and white, Age— $3\frac{1}{2}$  Years.  
Condition—Fair. Breed—Country.

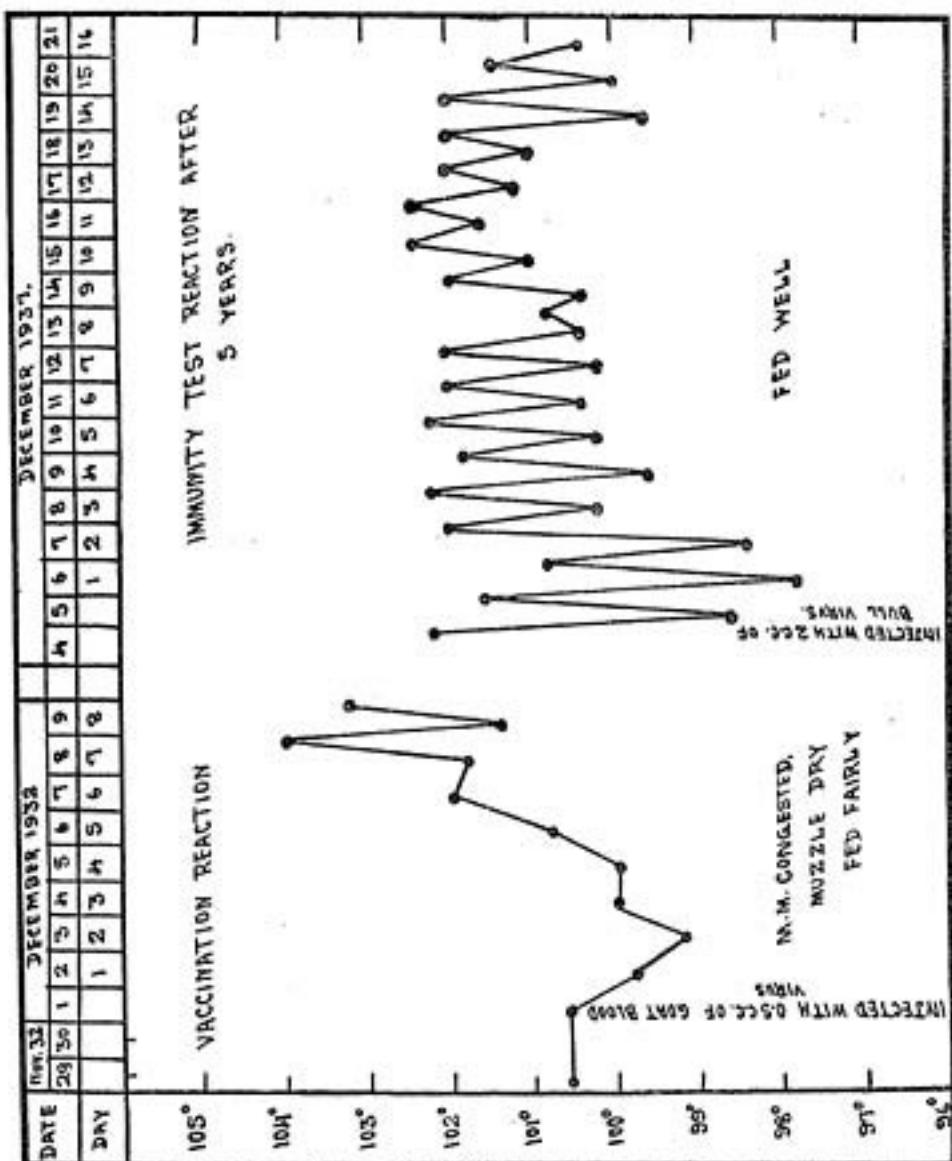


Fig. 3.  
Bullock, Paithan. Age—9½ Years, Condition—Good, Breed—Country.

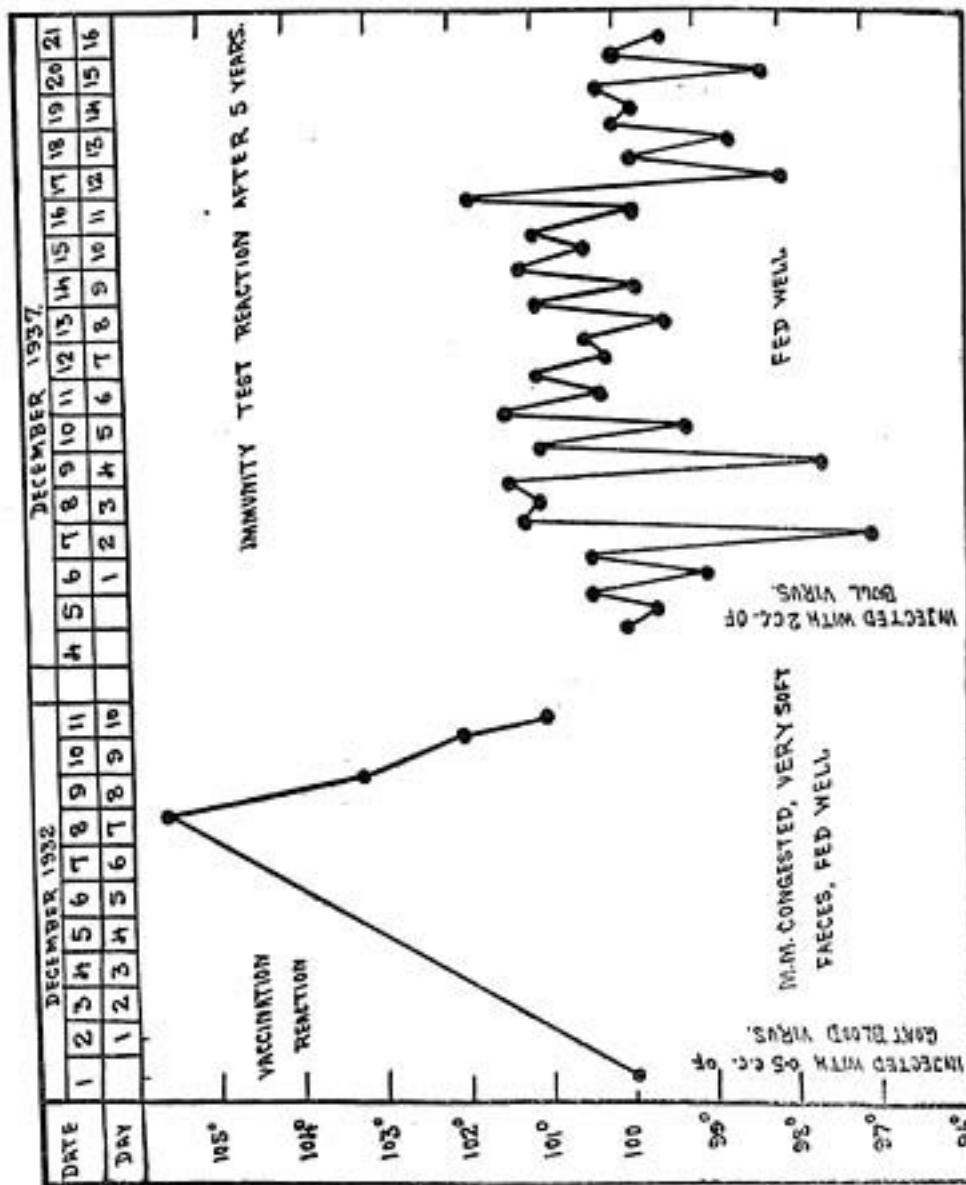


Fig. 4.  
Normal Cow. Gujjar : Age—8½ Years, Condition—Good, Breed—Delhi.

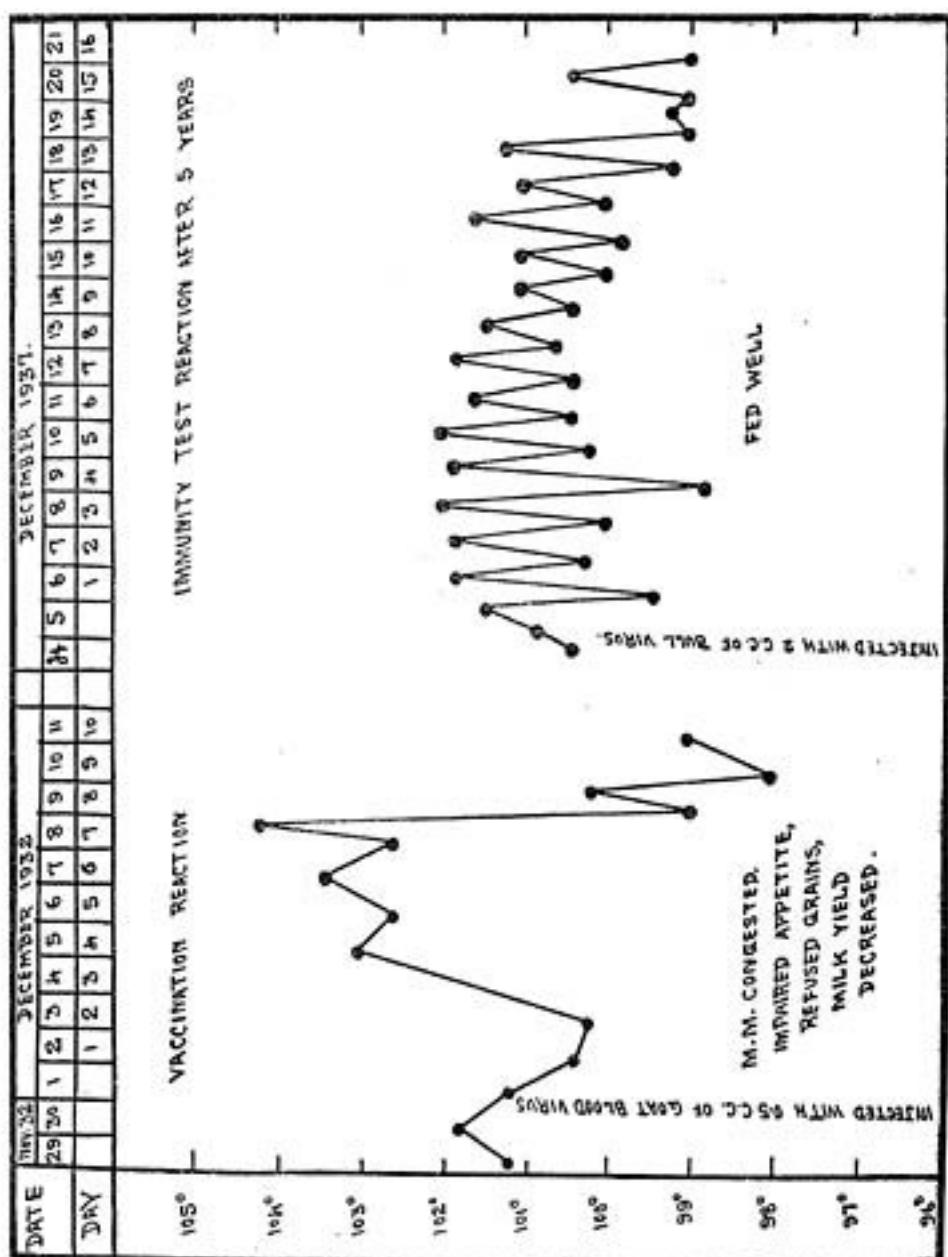


Fig. 5.  
Cow, Gawali : Pregnant 8 months, Ago—10 Years, Condition—Good, Breed—Country+

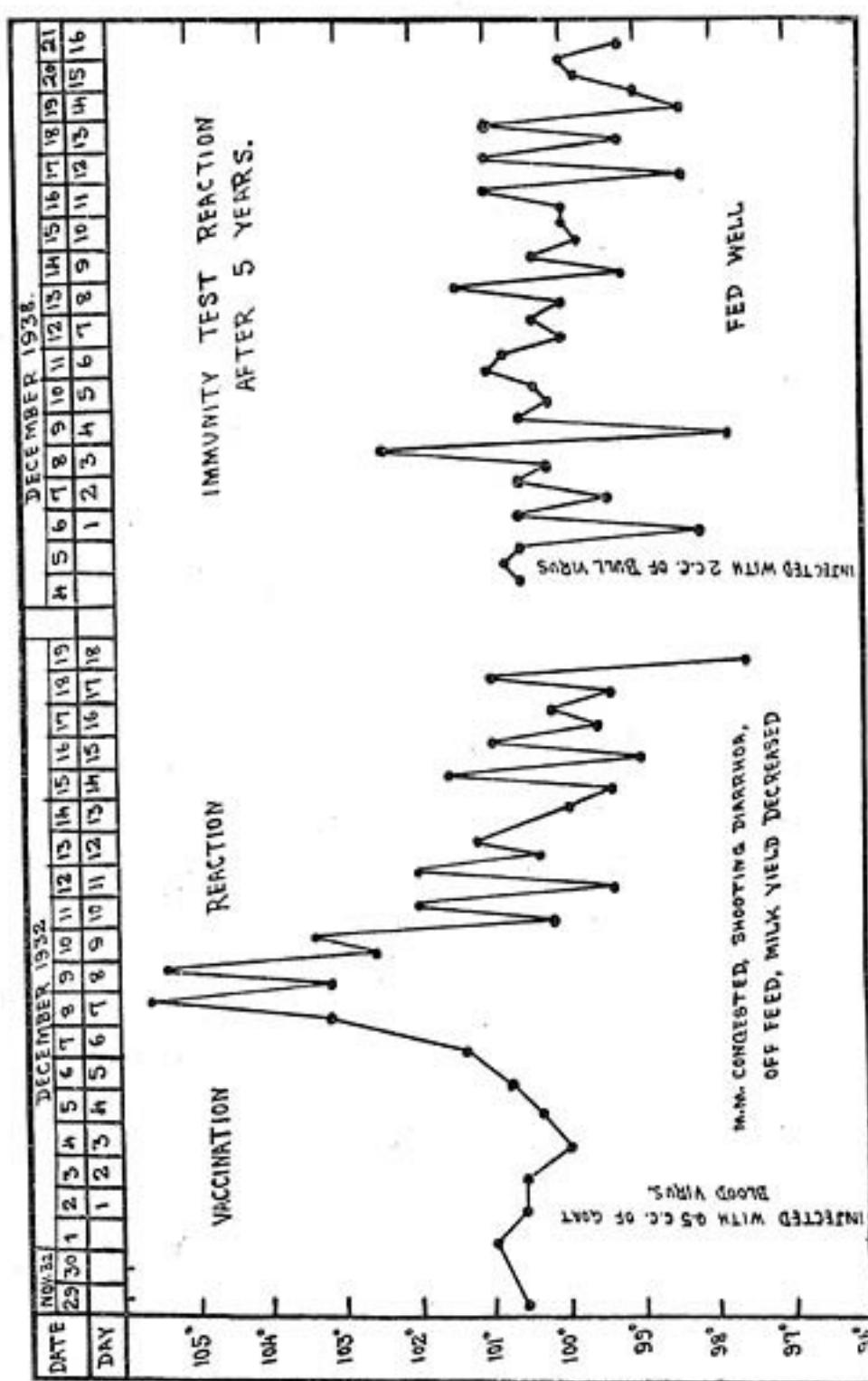


Fig. 6.  
Buffalo-Cow, Shobha; Milking; Age—10 Years, Condition—Good, Breed—Delhi.

TABLE I

| No. | Species and name of animal | Breed        | Age in yrs. | Details recorded during vaccination five years ago |                     |                    |  | Details recorded during immunity test |                                  |                                   |                        | Result               |
|-----|----------------------------|--------------|-------------|--|---------------------|--------------------|--|---------------------------------------|----------------------------------|-----------------------------------|------------------------|----------------------|
|     |                            |              |             | Condition and other history                        | Date of vaccination | Dose of goat-virus | Reaction produced by goat-virus vaccination                                | Age in yrs.                           | Condition and other history      | Date of subduing to immunity test | Dose of virulent virus |                      |
| 1   | Buffalo-ox, Pal-thain.     | Country      | 4½          | Good, Used for draught purposes.                   | 1-12-32             | 0·5 e.c.           | Fever, mucous membrane congested, mucus fed easily.                        | 9½                                    | Good, Used for draught purposes. | 5-12-37                           | 2 e.c.                 | No reaction          |
| 2   | Buffalo-ox, Gajjar.        | Gross, Delhi | 3½          | Fair, Pregnant 2 months.                           | 1-12-32             | 0·5 e.c.           | Fever, mucous membrane congested, very soft faeces.                        | 8½                                    | Good, pregnant month.            | 5-12-37                           | 2 e.c.                 | Do.                  |
| 3   | Cow, Gauri                 | Country      | 6           | Good, milking, pregnant 2 months.                  | 1-12-32             | 0·5 e.c.           | Fever, mucous membrane congested, impaired appetite, milk-yield reduced.   | 10                                    | Good, pregnant 3 months.         | 5-12-37                           | 2 e.c.                 | Do.                  |
| 4   | Buffalo-ox, Shroka.        | Delhi        | 5           | Good, milking, pregnant 1 month.                   | 1-12-32             | 0·5 e.c.           | Mucous membrane congested, off feed, diarrhoea, mucus, milk-yield reduced. | 10                                    | Good, milking.                   | 5-12-37                           | 2 e.c.                 | Do.                  |
| 5   | Controls.                  | Country      | -           | -  | -                   | -                  | -  | 2                                     | Fair                             | 5-12-37                           | 2 e.c.                 | Reaction impossible. |
| 1   | Bull                       | -            | -           | -  | -                   | -                  | -  | -                                     | -                                | -                                 | -                      | Do.                  |
| 2   | Bull                       | -            | -           | -  | -                   | -                  | -  | 2½                                    | Fair                             | 5-12-37                           | 2 e.c.                 | Do.                  |

It is observed from Table I that both oxen and buffaloes failed to contract rinderpest even after artificially infected with a massive dose of virulent virus ; pregnant animals showed no signs of ill-health to the foetus *in utero*, and the buffalo-cow in milk continued to yield normal quantity of milk. The two control bulls, on the other hand, developed rinderpest and as a sequel, there was resuscitation of *B. bigeminum* in one and of *T. mutans* in the other. The bull which had the complication of *B. bigeminum* was, at one time, considered likely to succumb on account of bloody urine it passed and the presence of a teeming number of the protozoa in the peripheral blood-film. But it made a spontaneous recovery along with its companion without any treatment.

#### SUMMARY

Four cattle which were vaccinated with goat-blood-virus five years ago were artificially infected with virulent rinderpest-virus with the result that every animal was found to possess solid immunity against rinderpest, thus showing that the duration of immunity conferred by goat-blood-virus vaccination is more than five years.

#### ACKNOWLEDGMENT

The work was carried out as a part of the duty of the writer whose budget is partly subsidised by the Imperial Council of Agricultural Research, New Delhi. The writer desires to express his indebtedness to Mr. E. S. Farbrother, M.R.C.V.S., I.V.S., Director of Veterinary Services, Bombay Presidency, for his valuable guidance in this work. Thanks are due to the authorities of the Ramabai Mukti Mission, Kedgaon, for the facilities they afforded in conducting this experiment.

## HELMINTHOLOGY IN RELATION TO VETERINARY SCIENCE

BY

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THE study of parasitic worms is technically known as Helminthology. Veterinary helminthology aims at the conservation of public and animal health. For a long time stockowners sincerely questioned the importance of worms and denied that they ever did any appreciable damage. They were inclined to attribute the losses due to worms to other causes as the common symptoms of helminthiasis—a prolonged and progressive afebrile unthriftiness gradually resulting in death—are not always as spectacular as are those of bacterial or virus infections. This lack of appreciation of the magnitude of the subject has been probably due to the fact that at times apparently healthy animals harbour an appreciable number of one or more species of worms. Helminthiasis, in common with other forms of parasitism, is a balanced relationship between the parasites and the hosts; and so long as the balance is maintained, no appreciable damage is apparent. When, however, on account of faulty nutrition, ingestion of poisonous substances, bacterial or virus infections or other causes, the vitality of the host is impaired, this balance is disturbed, with the consequence that the parasite gets the upper hand and it becomes increasingly difficult for the host to resist successfully the parasitic, bacterial or virus infections.

In recent years, there has been a gradual realization of the fact that, in a tropical country like India where animal hygiene is little developed, helminthiasis is a most serious menace to the health of livestock. Though the effects of helminths on their hosts may not always be sufficiently pronounced to attract immediate attention, they are responsible for enormous losses resulting from poor health and decreased resistance to diseases. Referring to the loss due to helminths, Cameron in 1932 remarked, "We are certainly underestimating the situation if we say that over ten million pounds are lost to this country (England) yearly through the agency of helminths. This estimate is based on the present low price of stock". Le Roux in 1930 pointed out that, in the Union of South Africa, thousands of animals died annually as a result of worm infestations. The worms alone contributed much towards swelling the ranks of the poor and towards driving men off the land. At several places the helminths rendered the profitable running of sheep as a side-line a practical

\* Presented to the Section of Veterinary Research of the Indian Science Congress 1938, Calcutta.

impossibility. Further, he pointed out that the annual heavy losses from the actual deaths of the parasitised represented only a very small fraction of the damage suffered from loss of condition and reduced productivity.

Helminths cause damage to the host in any one or more of the following ways :—

- (a) depriving the host of its food,
- (b) feeding on the tissues of the host,
- (c) mechanically causing obstruction or pressure,
- (d) causing abrasion through which secondary infection may take place,
- (e) mechanical irritation,
- (f) formation of nodules or tumours, and
- (g) elaboration of substances injurious to the host.

Though the pathogenicity of such helminths as give rise to some of the important conditions like distempsis, parasitic gastritis, verminous pneumonia, strongylosis and ancylostomiasis, etc., is readily recognised, there is still a large number the pathogenicity of which is not known. The association of large numbers of worms with pronounced and persistent poor condition of animals can be rightly interpreted only if the pathogenicity of the parasites concerned is known. The following are some of the important helminthic diseases of domestic animals :—

#### EQUINES

Horses are frequently so heavily infested with worms that they have been regarded as helminthological museums. Of the several kinds of worms infesting horses strongyles are the most important. Some of the strongyles are blood-sucking, but all of them tend to produce unthriftiness, often resulting in cachexia. It is a common experience of horse-owners that, when horses infested with worms are treated, they improve considerably in weight and condition. Foals when free from parasites have been reported to weigh hundred pounds more than those of the same age but infested with worms. Habronemiasis, both gastric and cutaneous, is another helminthic disease which is often responsible for much loss of condition, specially in the tropics. The eggs of *Schistosoma indicum* are frequently found associated with a peculiar form of nodulated hepatic cirrhosis, resulting in intractable debility in equines. The important manifestations of equine microfilariasis are the lichen tropicus or *khojlee* and periodic ophthalmia in horses.

#### RUMINANTS

Amongst the ruminants the most important and serious diseases are fascioliasis and parasitic gastritis. The annual loss due to liverflukes alone in ruminants has been estimated at \$1,000,000 in America and at a million pounds in England. The usual parasite responsible for producing gastritis in Indian

sheep and goats is *Haemonchus contortus* and in cattle *Mecistocirrus digitatus*. Fourie [1931] compared the anaemias of haemonchosis in sheep to that caused by daily withdrawal of blood from the jugular vein and found the two anaemias to be haematologically identical. In 1934, Martin and Ross attempted to estimate the amount of blood withdrawn by *Haemonchus contortus* from the amount of phosphorus eliminated with the eggs of the worms. It was found that even in moderate infestations about 30 c.c. of blood was used daily by the worms. This is the minimal figure and probably the actual amount would be several times more. Apart from the larvae of certain roundworms which pass through the lungs during their course of migration in the body, there are several worms which have their final habitat in the lungs, and produce marked pulmonary symptoms. Lungworms are specially dangerous in calves, sheep and goats. Another worm which is particularly dangerous to young animals is the ascarid parasite of calves and foals. Though, in the adult stage, amphistomes are practically harmless, they are highly pathogenic in their immature stages which are passed in the duodenum and intestine. The immature stages of amphistomes, specially of *Cotylophoron cotylophorum*, are often responsible for alarming symptoms, such as persistent foetid diarrhoea, oedema of the submaxillary space, intestinal haemorrhage and general unthriftiness, etc., and consequent high mortality. Recently, two other important diseases of bovines have been found to be due to helminths. They are nasal schistosomiasis and cutaneous microfilariasis. Besides the above, whipworms, intestinal nodule worms and tapeworms are often responsible for much damage to ruminants.

#### POULTRY

Helminths do considerable damage to poultry. Instances are not rare when most of the birds in a flock are lost as a result of worm infestations. The most harmful trematode of poultry is the one—*Prosthogonimus* sp.—found in the oviducts of birds. The worm, which usually lives in the Bursa fabricii, enters the oviduct in laying birds and is responsible for acute inflammation resulting in the production of abnormal eggs and discharge of albumen. Owing to irritation, retroperistaltic movements are set up in the oviduct causing broken eggs, egg contents and parasite material to enter the peritoneal cavity and giving rise to acute peritonitis which is often fatal. The eggs, if laid, are soft-shelled. Birds are often infested with large numbers of tapeworms of varying degree of pathogenicity. Taeniasis in poultry may be responsible for marked enteritis, rapid wasting and death. *Railietina tetragona* and *R. echinobothrida* cause the formation of nodules resembling those of tuberculosis. Apart from causing general loss of condition due to haemorrhage and enteritis, the large roundworms of poultry cause shrinking of the thymus gland and a decrease in blood sugar, increasing thereby the susceptibility of birds to certain infectious diseases, such as roup. The gapes are most dangerous to chicks, in which they give rise to dyspnoea and asphyxia, besides progressive emaciation. The stomach worms of poultry produce marked irritation, inflammation, ulceration and nodules in the gizzard and proventriculus. The nodules at

times may become large enough to cause mechanical interference and obstruction to the passage of food. The gizzard may be weakened to such an extent that a rupture may result. Stomach worms are reported to produce toxins as well.

#### CANINES

Amongst the important helminth parasites of the dog may be mentioned hookworms, tapeworms, ascarids, liver and lungflukes and *Spirocerca sanguinolenta*. Ancylostomiasis is probably the most important of all the helminthic diseases of the dog. Its chief symptom is anaemia due to loss of blood. Wells [1931] was able to observe living hookworms feeding on the mucous membrane in a loop of the small intestine of an anaesthetised dog. He observed that the hookworm fills the intestine with blood and ejects it through the anus at intervals varying from a few seconds to fifteen minutes. He computed that the quantity of blood removed by a single worm in 24 hours amounted to 0.85 c. c. In a heavy infestation the dog may harbour several hundreds of worms and consequently lose a large quantity of its blood daily.

Besides the helminths which infest domestic animals exclusively, there are a large number of worms which are common to man and animals either in their adult or larval stages. In such cases animals usually serve as the reservoir hosts. All such worms are important from a public health point of view and may be considered under three main heads :—

#### A. PARASITES WHICH LIVE AS ADULTS BOTH IN MAN AND ANIMALS

Examples of such parasites are the broad fish-tapeworm, the human whipworm, the dwarf tapeworm (*Hymenolepis nana*), the large intestinal fluke (*Fasciolopsis buski*), the lungflukes and the various members of the families Opisthorchiidae and Heterophyidae. *Dipylidium caninum* is a very common tapeworm of dog and cat, and is found not infrequently in children. Recently, in a series of papers, Africa, Leon and Garcia [1935 and 1936] have reported the occurrence of heterophyid eggs in the myocardium and the central nervous system of human beings and the fatal consequences due to acute vascular changes in the affected organs. In addition to the above there are a large number of helminths of domestic animals which occur as accidental parasites in man.

#### B. PARASITES WHICH LIVE AS ADULTS IN MAN AND AS LARVAE IN ANIMALS

Through eating improperly cooked, measly pork or beef, man gets infested with *Taenia solium* and *T. saginata* respectively. Besides the constitutional disturbances which those tapeworms cause in man, the former is dangerous because its larval stage can develop both in pig and man. It is found not only in the musculature but also in the central nervous system. Quite a number of cases of nervous derangements have been attributed to it. Perhaps the most important worm from a public health point of view is the trichina worm,

*Trichinella spiralis.* The infestation is acquired by eating raw or improperly cooked pork containing the encysted larvae. Heavy infestations are often fatal.

#### C. PARASITES WHICH LIVE AS LARVAE IN MAN AND AS ADULTS IN ANIMALS

The most important disease under this group is the well-known 'Hydatid' caused by the bladder worm stage of *Echinococcus granulosus* of dogs, cats and wild canidae. The intermediate hosts of this tapeworm are legion, almost any mammal. There are a large number of trematodes of birds and mammals, the cercariae of which actively penetrate into the skin and produce a marked local reaction known as cercarial dermatitis. Analogous to this is the condition known as 'Creeping eruptions' which is caused by a variety of parasites of lower animals penetrating into the skin of man and finding the environment unsuitable, move about in or under the skin. The commonest of such parasites is the immature hookworm of dogs and cats.

Apart from the parasites which are injurious to man in one way or other, there are several which by their presence render meat unfit for human consumption, e.g., liverflukes, tapeworms, hydatid cysts, kidney worm and *Onchocerca* sp. in muscles, etc.

Though there are at present no statistics to show the actual economic loss due to helminths in this country, few will deny the importance of helminths as factors determining the health of stock. The problems of veterinary helminthology are highly complex. These parasites are highly specialised and have acquired a high degree of adaptation to their peculiar modes of life. The relations existing between them and their hosts are in all respects governed by their life-history. A detailed knowledge of their life-histories is necessary to determine the nature and extent of the pathological conditions to which they give rise or to devise means of protection against them. As development of science makes it possible to keep more and still more animals on a limited area, so also does it make an increase in the number of their parasites possible. Unless helminth parasites are studied systematically and thoroughly, the list of obscure and undiagnosed diseases, puzzling anaemias and mysterious enlargement of organs will remain lengthy and will continue to be a stumbling block in the conservation of animal health in this country.

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# ESTIMATION OF TOTAL SULPHATE IN BLOOD SERUM

BY

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TOTAL sulphates are usually estimated by hydrolysing the substance and precipitating the sulphates as barium or benzidine salts. The nephelometric method of Denis and Reed [1926-1927] suffers from the disadvantage that the turbidity of the suspension of barium sulphate is dependent on changes in the pH and composition of the blood filtrate.

Wakefield [1929] hydrolysed blood filtrate with hydrochloric acid and estimated the sulphates as benzidine sulphate. He used two drops of concentrated hydrochloric acid to 5 c.c. of blood filtrate and kept the tube in boiling water for fifteen minutes. It was allowed to cool and benzidine solution was then added. The present author has found that with blood serum of cattle, the concentration of acid used and the time allowed for hydrolysis are not sufficient for complete hydrolysis. The hydrochloric acid present is also partly precipitated as benzidine chloride, which being insoluble in acetone cannot be removed by washing. Fiske [1921] studied the difficulties which are encountered when benzidine is used to separate sulphate quantitatively from other inorganic compounds such as phosphates and chlorides. Hubbard [1930] also mentioned about the interference of benzidine chloride when sulphate was precipitated in the hydrolysed liquid.

The present work was started to find out a method of avoiding these difficulties and to work out a suitable technique for the estimation of total sulphates in blood serum.

## I. EXPERIMENTAL WORK

*Hydrolysis.*—Tests were made to find out the effect of the concentration of hydrochloric acid and the duration of boiling on hydrolysis of protein-free serum filtrates.

(a) *Effect of the concentration of acid.*—Duplicates did not agree in many cases when lower concentrations of acids, viz., 0·1 c.c. conc. hydrochloric acid in 5 c.c. filtrate were used for hydrolysis. Better results were obtained with higher concentrations. Two c.c. serum filtrate samples were hydrolysed for two hours with different quantities of conc. hydrochloric acid 0·1 c.c. acid always gave slightly lower results. 0·2 c.c. acid was also sometimes insufficient for complete hydrolysis. Good results were obtained with 0·3 c.c. acid but as the presence of more acid during hydrolysis did not interfere with the sulphate precipitation, if the technique explained in the sequel were followed, the writer preferred 0·4 c.c. acid to avoid any risk of incomplete hydrolysis.

TABLE I  
*Effect of the amount of acid on hydrolysis*

| Amount of conc. hydrochloric acid used for 2 c.c. filtrate | Mg. per cent S as total sulphate |          |      |      |
|--|----------------------------------|----------|------|------|
|  | Sample 1                         | Sample 2 |      |      |
| 0·1 c.c. . . . . . . .                                     | 3·94                             | 4·81     | 5·49 | 5·56 |
| 0·2 c.c. . . . . . . .                                     | 5·44                             | 5·10     | 5·68 | 5·81 |
| 0·3 c.c. . . . . . . .                                     | 5·40                             | 5·44     | 5·75 | 5·81 |
| 0·4 c.c. . . . . . . .                                     | 5·44                             | 5·40     | 5·81 | 5·75 |
| S found by the macro method . . . .                        | 5·59                             |          | 5·96 |      |

Table I shows that the extent of hydrolysis obtained with 0·3 c.c. and 0·4 c.c. acid compares favourably with that obtained by the macro method, where 400 c.c. of serum filtrate, equivalent to 80 c.c. serum, were hydrolysed with 20 c.c. concentrated hydrochloric acid and the sulphate was precipitated and weighed as barium sulphate.

(b) *Effect of time on hydrolysis.*—The effect of the duration of hydrolysis with the same concentration of acid, 0·4 c.c. for 2 c.c. filtrate, was tested with some samples. The following results in Table II show that half hour and one hour boiling gave lower results. There was no difference between two hours and four hours' boiling. Therefore even with this increased concentration of acid, blood filtrate samples should be hydrolysed for at least two hours to ensure complete hydrolysis.

TABLE II  
*Relation between time and amount of hydrolysis*

| Duration of hydrolysis        | Mg. per cent S as total sulphate |          |          |      |
|-------------------------------|----------------------------------|----------|----------|------|
|                               | Sample 1                         | Sample 2 | Sample 3 |      |
| Half hour . . . . .           | 4·63                             | 4·90     | 5·49     | 2·81 |
| One hour . . . . .            | 4·90                             | 5·56     | 5·10     | 2·38 |
| Two hours . . . . .           | 5·44                             | 5·95     | 5·75     | 2·84 |
| Four hours . . . . .          | 5·40                             | 5·81     | 5·75     | 2·84 |
| S found by the macro method . | 5·59                             | 5·96     |          | 2·91 |

## 2. PRECIPITATION OF BENZIDINE SULPHATE

(a) *Interference caused by the presence of hydrochloric acid.*—It was found that large quantities of chlorides separated when 5 c.c. of 0·5 per cent benzidine in acetone was added to 2 c.c. of the hydrolysed sample. Even if one drop of concentrated hydrochloric acid is used for hydrolysis, higher results are obtained on account of the interference of chlorides. Sulphates estimated in the same sample after removal and without removal of the drop of hydrochloric acid used for hydrolysis gave 3·05 per cent and 4·3 per cent S respectively. In some cases when the quantities of acid were small, it was noticed that the chlorides might not separate with the addition of the benzidine solution. It remained in solution in the liquid (about 70 per cent acetone by volume). But, while washing, after the first decantation, the traces of the chloride solution left adhering to the sulphate precipitate or sticking to the sides of the tube became insoluble as soon as acetone was added for washing the precipitate. Higher concentrations of hydrochloric acid kept both benzidine sulphate and benzidine chloride in solution, the solubility increasing with the hydrochloric acid concentration.

This difficulty was overcome by frequent tests in this laboratory which showed that if all the free hydrochloric acid was removed by evaporation in vacuum at 100°C, the quantities of chlorides left in the hydrolysed sample did not interfere with the results when pure acetone was used for washing.

TABLE III

*Relation between concentration of hydrochloric acid and precipitability of benzidine salts*

| Concentration of hydrochloric acid      | Mg. per cent S as total sulphate |
|---|----------------------------------|
| No hydrochloric acid . . . . .          | 5·44                             |
| 0·25 c.c. N hydrochloric acid . . . . . | 5·96                             |
| 0·5 c.c. N hydrochloric acid . . . . .  | 5·81                             |
| 1·0 c.c. N hydrochloric acid . . . . .  | 4·67                             |
| 1·8 c.c. N hydrochloric acid . . . . .  | 0·61                             |

Two c.c. serum filtrate samples were hydrolysed and evaporated to dryness in vacuum to remove the hydrochloric acid used for hydrolyses. 0·2 c.c. of 20 per cent tri-chloroacetic acid and different quantities of N hydrochloric acid were added to the residues and the volumes made up to 2 c.c. Five c.c. benzidine in acetone were added to each sample. Table III shows that higher results were obtained when either 0·25 c.c. or 0·5 c.c. N hydrochloric acid was present due to the precipitation of benzidine chloride. One c.c. N hydrochloric acid kept some benzidine sulphate in solution. No precipitate

separated with 1.8 c.c. acid, the solution in the tube was quite clear; the figure obtained, viz., 0.61 mg. S, was due to the soluble benzidine salts adhering to the sides of the tube made insoluble by the pure acetone used for washing.

(b) *Effect of acidity of the liquid on the benzidine sulphate precipitate.*—The trichloracetic acid present in the serum filtrate was also removed along with the hydrochloric acid used for hydrolysis when the hydrolysed sample was dried under vacuum. The dried substance left in the tube was practically neutral. This was dissolved in water and several attempts were made to precipitate sulphate in this neutral solution but they were not successful. The liquid should be acidic. The following table shows the optimum acidity required for the complete precipitation of benzidine sulphate:—

TABLE IV

| Amount of acid added to the dried sample | Mg. S per cent as total sulphate |                |                |   |                |
|--|----------------------------------|----------------|----------------|---|----------------|
|  | Sample A                         | Sample B       | Sample C       | Sample C<br>+<br>0.01 mg.<br>phosphorus |                |
| 2 c.c. water; solution neutral           | No precipitate                   | No precipitate | No precipitate |   | No precipitate |
| 2 c.c. 0.5 per cent trichloracetic acid  | ..                               | 10.00          | 9.62           | ..                                      | ..             |
| 2 c.c. 1 per cent trichloroacetic acid   | ..                               | ..             | ..             | 2.78                                    | ..             |
| 2 c.c. 2 per cent trichloroacetic acid   | 3.33                             | 5.26           | 5.20           | 2.79                                    | 2.78           |
| 2 c.c. 3 per cent trichloroacetic acid   | ..                               | ..             | ..             | ..                                      | 2.84           |
| 2 c.c. 4 per cent trichloroacetic acid   | 3.26                             | 5.10           | 5.10           | 2.29                                    | ..             |
| By macro method                          | 3.50                             | 5.15           | 2.91           | 2.91                                    | 2.91           |

Five c.c. of 0.5 per cent benzidine in acetone was added for precipitation. There was no precipitate when the solutions were neutral. But sulphates separated as soon as the solution was made acid with one drop of 20 per cent trichloroacetic acid. The above figures show that best results were obtained when 2 c.c. of 2 per cent acid was used. Four per cent acid gave decidedly lower results.

(c) *Interference caused by the presence of phosphates.*—The results obtained with less acid in Table IV are of interest. With sample B much higher results (100 per cent) were obtained when the liquids were just acid. Similarly in the case of sample C, one per cent acid gave

good results with the normal sample, viz., 2.78 mg. S per 100 c.c. serum, but when the inorganic phosphorus content was nearly doubled by the addition of 0.01 mg. phosphorus in each tube (1 c.c. serum filtrate equivalent to 0.2 c.c. serum was taken for each estimation in the case of sample C. 0.01 mg. extra phosphorus in 0.2 c.c. serum is equivalent to 5 mg. extra phosphorus per 100 c.c. serum), one per cent acid figures were 7.81 and 6.25 mg. S per cent, that is more than double the quantity present in the sample. The higher results in both the samples B and C were due to the separation of benzidine salts of phosphoric acid on account of insufficient acid. High concentration of acid tended to hold benzidine sulphate in solution and low ones to permit the precipitation of phosphates. With samples of normal phosphorus content (5 mg. inorganic phosphorus per cent) one per cent acid might be sufficient in certain cases to keep the phosphates present in solution, but with samples of abnormally high phosphorus content it was safer to use three per cent acid.

The following observations in Table V confirm the results obtained in Table IV :—

TABLE V

| Mg. phosphorus in the tube | Acidity of the 2 c.c. phosphate solution in terms of per cent trichloracetic acid |                    |                    |                |
|----------------------------|---|--------------------|--------------------|----------------|
|                            | Neutral   | One per cent       | Two per cent       | Three per cent |
| 0.01 . . .                 | No precipitate  | Slight precipitate | No precipitate     | No precipitate |
| 0.02 . . .                 | No precipitate  | Precipitate .      | No precipitate     | No precipitate |
| 0.08 . . .                 | No precipitate  | Precipitate .      | Slight precipitate | No precipitate |

Pure potassium phosphate solutions were used for the above tests. Five c.c. of 0.5 per cent benzidine in acetone were added to each tube. The table shows that, like sulphates, phosphates also did not precipitate in neutral solutions. Two per cent acid was not sufficient when 0.08 mg. phosphorus was present.

(d) *Time required for the quantitative precipitation of sulphate.*—Cuthbertson and Tompsett [1931] centrifuged the solution half-an-hour after the precipitation. Pirie [1934] recommended at least two hours in ice for complete precipitation. Trials here showed that better results were obtained when the samples were kept overnight in the refrigerator.

(e) *Recovery of added organic sulphates.*—The results obtained by following the technique suggested have been frequently checked by duplicate analyses with different quantities of serum filtrate and by gravimetric methods. Further, to test the accuracy and reliability of the method, minute quantities of benzidine sulphate solution in hydrochloric acid were

added to serum filtrate, which were then estimated as usual. Blank determinations on the serum filtrate alone were made simultaneously. The results obtained are given in Table VI.

TABLE VI

| —              | Mg. S added to serum filtrate | Mg. S found | Excess over blank |
|----------------|-------------------------------|-------------|-------------------|
| Sample A . . . | 0                             | 0·0244      | ....              |
|                | 0·002                         | 0·0265      | 0·0021            |
|                | 0·004                         | 0·0285      | 0·0041            |
|                | 0·006                         | 0·0301      | 0·0057            |
| Sample B . . . | 0                             | 0·0253      | ....              |
|                | 0·002                         | 0·0274      | 0·0021            |
|                | 0·004                         | 0·0294      | 0·0041            |
|                | 0·006                         | 0·0313      | 0·0060            |

The figures show that added sulphates could be accounted for satisfactorily.

As a result of the above tests, the following method was found to give quite satisfactory results for total sulphates. Two c.c. serum are diluted with 6 c.c. water, 2 c.c. of 20 per cent trichloracetic acid added, mixed thoroughly and filtered through a dry ashless filter after fifteen minutes. Two c.c. filtrate are taken in a 15 c.c. centrifuge tube and hydrolysed with 0·4 c.c. conc. hydrochloric acid for two hours in a beaker of boiling water. After the hydrolysis the liquid is evaporated to dryness in vacuum at 100° C. The residue is dissolved in 2 c.c. of 2 per cent trichloracetic acid and sulphate precipitated with 5 c.c. of 0·5 per cent benzidine in acetone. After mixing thoroughly the tube is placed in a wide mouth stoppered bottle and kept in the refrigerator.

Next day the precipitate is washed and sulphate estimated colorimetrically. The method of Cuthbertson and Tompsett [1931] was found quite satisfactory for this purpose. The addition of 15 per cent sodium hydroxide was omitted as recommended by Pirie [1934].

#### SUMMARY

1. A method had been described for the determination of total sulphate in blood serum.

2. The hydrochloric acid which usually interferes with the estimation of total sulphates is removed by vacuum evaporation.
3. The optimum acidity required for the quantitative precipitation of sulphates and for keeping the phosphates in solution has also been studied.

#### ACKNOWLEDGMENTS

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*DIPETALONEMA DRACUNCULOIDES* (COBBOLD, 1870)

BY

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[With three text-figures]

THE presence of *Dirofilaria repens* Railliet and Henry [1911] in the subcutaneous tissue of dogs in Bihar and Orissa has been recorded by Panda [1932] and *Dirofilaria immitis* [Leidy, 1856] has been recorded in indigenous dogs in South Bengal by Mitter [1912]. As regards *D. immitis*, it would appear from the references cited by Bhalerao [1935], that in other places in India, it has been recorded only in imported dogs particularly from Burma and Federated Malay States. Hence Mitter's finding of *D. immitis* in indigenous dogs of South Bengal may require confirmation. So far as Madras Presidency is concerned, these two species of worms have not been found in indigenous dogs hitherto. The embryos of these two species, however, are found in the circulating blood.

Rao [1923] and Korke [1924] described *Haematozoon lewisi* (*Filaria reccondita* Grassi) and *Microfilaria lewisi*, respectively, in the circulating blood of dogs, but neither of them could find any adult filarial worms in the blood vessels and other organs at *post mortem* examination. No worker in India has so far recorded *Dipetalonema dracunculoides* [Cobbond, 1870] in dogs. The present opportunity is, therefore, taken to record its presence in this country. The embryos of this species are found in the circulating blood and resemble very closely the microfilariae described by Rao [1923]. The description of *Microfilaria lewisi* given by Korke [1924] conforms to that of *D. dracunculoides*. Korke himself admits this fact and further adds that it is probable that the microfilariae so frequently observed by Lewis in Calcutta (and whose measurements as mentioned by Mitter [1912] were shorter than those of the embryos of *D. immitis*) are identical with *Microfilaria lewisi*. It is, therefore, apparent that both Rao and Korke described the same species of microfilariae which are actually the embryos of *D. dracunculoides*, and this is confirmed by the finding of the adult worms in the peritoneal cavity of an indigenous dog of the Rajapalayam breed of South India. The author obtained 18 males and 43 female worms from that dog.

*Description of the male worm.*—Length 22 to 27 mm.; breadth 0.140 to 0.200 mm. at its middle. Filiform, cuticle smooth, but in some it looked as if there are a few fine longitudinal straitions. The two extremities gradually taper, the head end being blunt and the tail end pointed. The tail is coiled spirally and has three to four coils. The end of the tail bears on it two lateral digitations giving it a trifid appearance, as in the genus *Setaria* [Viborg, 1795].

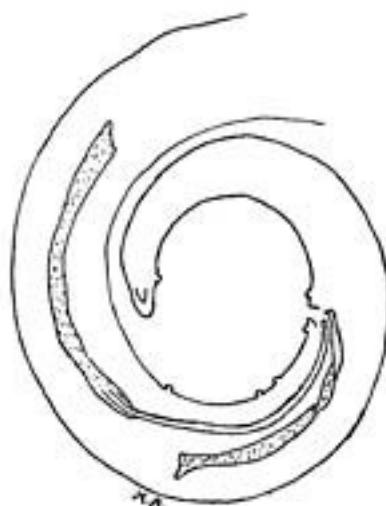


Fig. 1—Tail end of Male.



Fig. 2—Head end of Female.

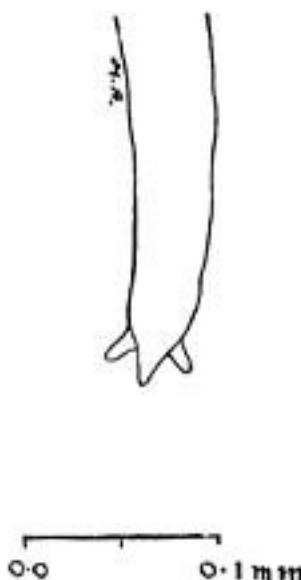


Fig. 3—Tail end of Female.

There are four pairs of pre-anal and two pairs post-anal papillae. These papillae are small and the cloacal opening is guarded by the last pair of pre-anal and the first pair of post-anal papillae. The second pair of the post-anal papillae is just in front of the lateral digitations. The spicules are unequal. The left one is long and is divisible into an anterior wide and cylindrical part and the posterior narrow and tubular one. It is approximately 0.310 mm. long. The right spicule, which is smaller than the other, measures nearly 0.161 mm. in length, is cylindrical and below its middle it is more or less twisted and then gets attenuated to terminate into a fine hook or a crochet hook as Railliet and his collaborators [1912] designate it. The head end is rounded. The mouth is a simple pore and is guarded by two lateral flattened papillae. There are four sub-median papillae on the head.

*Description of the female.*—Length 30 to 55 mm.; breadth 0.200 to 0.300 mm. at its middle. The head end is as in the male. (Fig. 2.) The tail end is also as in the male (Fig. 3) but not spirally coiled. In some of the immature females the tail end shows a tendency to coil spirally and usually there is a curve representing half a turn and rarely a full one. The vulva is situated in the region of the posterior half of the oesophagus, about 1.5 mm. from the head end. Opisthodelphys. Viviparous. Embryos unsheathed 200 to 225  $\times$  5 $\mu$  in length.

It will be thus seen that the description of the worms given above agrees with that of *Dipetalonema dracunculoides* [Cobbold, 1870] given by Railliet and his collaborators [1912].

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*POTERIOSTOMUM RATZI* (KOTLAN, 1919)

BY

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[With two text-figures]

ON examination of a collection of *Trichonema* obtained last year, two species of *Poteriostomum* were found, viz., *P. imparidentatum* [Quiel, 1919] and *P. ratzi* [Kotlan, 1919]. The former has been reported from the Punjab and United Provinces [Bhalerao, 1935]. The latter species has not been recorded hitherto in horses in India, and a brief description of it is given below. The infestation with these worms seems to be small and the writer found about six of the former and four of the latter species in the large intestines of two different hackney ponies in Madras. No dimensions of the worms are noted since there were only three males and one female available for study, and only the male worm is described below for obvious reasons.

*Male*.—Mouth collar is marked off from the body by a groove. The elements of the external leaf crown are slender and many, and the internal leaf crown is composed of elements of equal size and furnished with pointed ends (Fig. 1, see page 132). The formula of bursal rays is as follows:—

The main trunk of the dorsal ray is not cleft to its base but only to half its length and the two lateral branches arise from the undivided portions close to the place of origin of the externo-dorsal ray. The externo-dorsal ray originates from a common trunk with the dorsal (Fig. 2, see page 132).

*Host*.—Hackney pony.

*Habitat*.—Large intestines.

*Place*.—Madras.

REFERENCE

Bhalerao, G. D. (1935). Helminth Parasites of the Domesticated Animals in India. *Imp. Coun. Agri. Res. Sci. Mon.* No. 6, pp. 365.

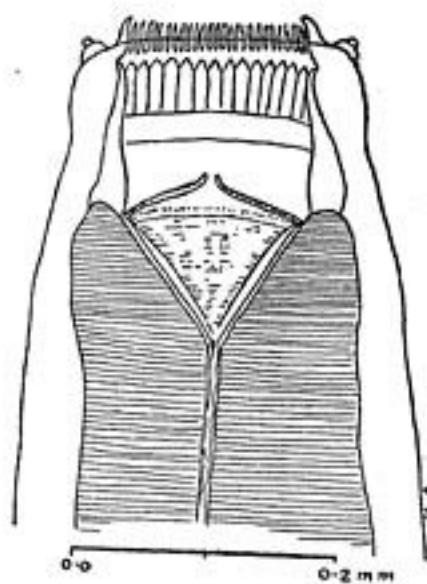


Fig. 1—Head end.

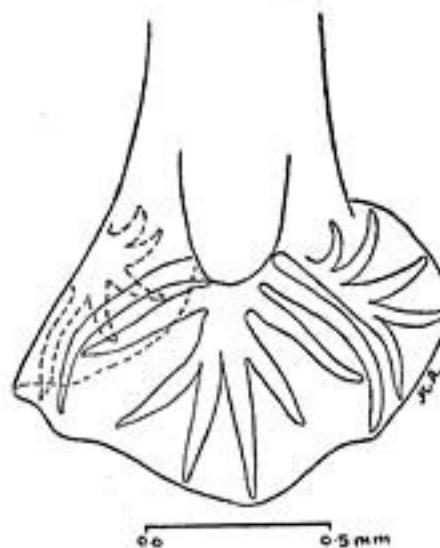


Fig. 2—Bursa of male.

# A CHECK- AND HOST-LIST OF IXODOIDEA (TICKS) OCCURRING IN INDIA

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## INTRODUCTION

A PERUSAL of the literature reveals that comparatively little work has been done to compile a check-list and host-list of the Ixodoidea (ticks), a highly interesting group of Arthropoda, many of which are of immense importance to human economy as being responsible for the transmission of various diseases amongst men and domesticated animals.

The Ixodidae occurring in this country have been very ably listed by Sharif [1928] who recorded altogether nine genera and forty-five species, four sub-species and six varieties, but the only reliable account of the Argasidae has been given by Nuttall, Warburton, Cooper and Robinson [1908-1926].

Since these publications, additional records have accumulated and it is felt that the data such as that given in the present paper concerning the distribution and hosts of the ticks (both Argasidae and Ixodidae) are worth publishing for ready reference of interested workers.

Almost all the new records that have come to light as a result of the present study are based on collections of the Imperial Veterinary Research Institute at Mukteswar (India), and the ticks dealt with were for the most part already determined by Warburton, Sharif and Sen (S. K.), excepting a few specimens examined by the author.

In arranging the species in the text, only the original references to the ticks concerned, together with the reference of either Nuttall *et al* "A Monograph of Ixodoidea" in case of the Argasidae, or Sharif's paper "A Revision of the Indian Ixodidae with special reference to the Collection in the Indian Museum" in case of the Ixodidae are given. The species of the various Indian ticks that are represented in the collection of the Imperial Veterinary Research Institute at Mukteswar are marked with asterisks (\*) in the host-parasite list appended. In discussing the distribution and hosts of the Ixodoidea only the records of the strictly Indian territories have been taken into consideration.

## DISTRIBUTION AND HOSTS

*I. Argasidae**Argas persicus* (Oken).

Oken [1818]. *Isis*, pp. 1567-1570; Nuttall, Warburton, Cooper and Robinson [1908]. *A Monograph of the Ixodoidea*, p. 20.

The species has been obtained from the following localities as exhibited in the Mukteswar collection:—

*Punjab*.—Capt. Greig collected some specimens from Kasauli in 1906 (Nuttall et al). The species has since been quite frequently recorded from fowls and pheasants in the Punjab.

*United Provinces*.—Bhim Tal and Allahabad off fowls and poultry, Mukteswar on bed (presumably can infest man), and Babugarh off dog.

*Central Provinces*.—Nagpur (?) off poultry.

*Bihar*.—Patna.

*North-West Frontier Province*.—Tarnab off fowl.

*Hyderabad*.—Off fowl.

*Bombay*.—Off fowl.

In South Africa the species is known to infest ducks, geese and turkeys. In Iran it attacks men.

*Ornithodoros savignyi* (Audouin).

Audouin, J.V. [1827]. "Description de l'Egypte.....le Grand", ed. 2, XXII, Zoologie, p. 183; Nuttall et al [1908]. *A Monograph of the Ixodoidea*, p. 45.

Christophers [1906] first recorded the species from South India. Additional records as shown from the collection at Mukteswar are as follows:—

Bombay from cattle shed, and Hyderabad (Sitarampet) off cattle.

The species has also been shown to attack man and Lounshury (according to Nuttall et al, he may have included *O. moubata* in the statement) reports that it feeds on fowl, dog, horse, goat, cattle and pig. It has been recorded from Aden off camels.

*Ornithodoros megnini* (Dugès).

Dugès, A. [1883]. *Naturaleza de Mexico*, V, p. 196; Sen, S. K. [1937]. *Ind. J. Vet. Sci. & Anim. Husb.* 7, 3, pp. 213-218.

Sen first reported the occurrence of the species from India, and it has been recorded from the following places:—

Central Provinces, Ahmednagar, and Saugor; Jubbulpore and Trimulgherry; the species being collected in each of these places off horses.

The species chiefly infests the ears of horse, ass, ox, and not infrequently human ear in Mexico (Nuttall et al). It is very common in the various parts of the United States.

*Ornithodoros lahorensis* Neumann.

Neumann, L. G. [1908]. *Arch. de Parasitologie*, XII, p. 17; Nuttall et al [1908]. *A Monograph of the Ixodoidea*, p. 69.

Montgomery first collected the ticks in 1906 from *Ovis aries* in Lahore. The species has since been reported from—

Sohawa (Punjab) off cattle and Peshawar (N.-W. F. P.) off sheep.

The species appears to be restricted to north-west part of India.

*Ornithodoros papillipes* Birula.

Birula, A. [1895]. *Bull. de l'Acad. Imp. des Sc. de St. Petersbourg*, ser. 5, II. (4), p. 354; Nuttall et al [1908]. *A Monograph of the Ixodoidea*, p. 80.

The species has been collected from Murree Hills and off dog and cattle in Punjab as shown in the collection at Mukteswar.

Birula's specimens were collected from Caucasus by Motschulsky. Neumann, however, considers this species a synonym of *O. tholozani*.

*II. Ixodidae**Ixodes acutitarsus* (Karsch).

Karsch, F. [1880]. *Mittheil d. Münchener entomol. Vereins*, Jahrg. IV, p. 142; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 236.

Neumann recorded the species from Sikkim and Abor country (India) in 1901 (Nuttall et al). The species has been taken off ox by Sharif in Miri Hills (Upper Assam), and Nuttall recorded its occurrence on a man from Salween Valley (Tibet).

*Ixodes ricinus* (Linn.).

Linnaeus [1746]. *Fauna suecica* No. 1142; *Idem* [1758]. *Systema Naturae*, 10th ed., p. 615; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 233.

The species has been known to prevail in India in the following places:—

Kashmir off dog, and Kangra Valley (near Kareri Lake) off sheep.

The species prevails almost all over the world and has been known to feed on a number of hosts such as cattle, horse, tiger, mouse, lizard, etc.

*Ixodes granulatus* Supino.

Neumann [1911]. *Das Tierreich*, XXVI, pp. 20-21; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 237.

This species has so far been recorded in India proper from the under-mentioned places:—

Pashok in Darjeeling District off *Epimys rufescens*.

Kobo in Abor country off *Sciurus erythraeus intermedius*.

Kohima in Naga Hills (Assam) off squirrel.

*Ixodes japonensis* Neumann.Neumann [1904]. *Arch. de Parasitologie*, VIII, pp. 458-459.

This species is being recorded for the first time in India from Mukteswar off a barking deer (*Cervulus aureus*) locally known as ' kakar '.

This species was created on the strength of one female specimen collected in Tokyo (Japan) by Harmand in 1901.

*Haemaphysalis seticelli* Sharif.Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 245.

Sharif created this species from two females taken at Abbottabad (N.-W. F. P.) off a goat.

*Haemaphysalis sundrui* Sharif.Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 246.

This species has been created by Sharif from three females collected by Sunder Rao at Bhowali (U. P.)

*Haemaphysalis montgomeryi* Nuttall.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 395-397 ; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 248.

The species has a wide range of distribution in the hilly areas of Northern India and has been known to parasitise domestic animals. The following records are taken from Sharif :—

*United Provinces*.—Bhim Tal, Bhowali.

*Punjab*.—Below Bhagu, Kasauli.

*Haemaphysalis flava* Neumann.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 408-410 ; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 250.

The species has been recorded from the following places :—

*United Provinces*.—Bhowali and Mukteswar ; Madras.

At Mukteswar collection from 1922 to 1926 the host-list showed to be the hill bull, dog, fox, jackal and man.

*Haemaphysalis turturis* Nuttall and Warburton.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 410-411 ; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 251.

Only males of the species are known and have been recorded from Parambikulam (Cochin) off Nilgiri wild goat, and Bhowali, (U. P.) off cattle.

*Haemaphysalis birmaniae* Supino.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 415-416; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 253.

The species is restricted to the Eastern Himalayas, the localities recorded by Sharif being Sadya in Lakhimpur District (Assam), and Pashok in Darjeeling District (Bengal) off deer.

*Haemaphysalis hystricis* Supino.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 422-426; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 254.

Sharif recorded the species for the first time in India from Abor country (Assam), Nagalberna in Goalpara District (Assam) off tiger, and Darjeeling District (Bengal).

*Haemaphysalis bispinosa* Neumann.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 426-433; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 257-258.

The species has been recorded from the various parts of India and from a number of domestic and wild animals such as cattle, goat, dog, buffalo, horse, tiger, deer, etc., as will be seen from Sharif's paper.

From the collection at Mukteswar, the only new records outside the provinces given by Sharif seem to be those of Mysore and Hyderabad.

*H. bispinosa* var. *intermedia* Warburton and Nuttall is not so widely distributed, being recorded from Bihar and Orissa, Central Provinces, United Provinces, Madras and Bombay. It usually attacks wild animals but has also been collected from cattle and dog (Sharif). The Mukteswar collection contains specimens of this variety taken off cattle.

*Haemaphysalis parva* Neumann.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 435-437; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 260.

The species has been known to prevail in Satpara in Puri District (Orissa), and the collection at Mukteswar shows its distribution to Mysore where it was taken off cattle.

*Haemaphysalis aculeata* Lavarra.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 440-442; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 261.

Sharif observed a single male specimen of this species from Bangalore (Mysore) which was taken off a man.

*Haemaphysalis spinigera* Neumann.

Neumann [1910]. *Ann. Sci. Nat.* (9), XII, pp. 174-175; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 263.

The occurrence of the species has been recorded by Sharif in certain localities under Bombay, Madras, Central Provinces and Orissa and the species was mostly found to be associated with bullock and buffalo.

*Haemaphysalis leachi* (Audouin).

Audouin [1827]. "Description.....le Grand", ed. 2, XXII, *Zoologie*, p. 428; Sharif [1928]. *Rec. Ind. Mus.* p. 264.

Sharif records the species from Bihar and Orissa off leopard and wolf (the latter was shot near Kodarma in Hazaribagh District).

*H. leachi* var. *indica* Warburton is more common in India, being recorded from Bengal, Madras, Bombay, Central Provinces, Orissa and United Provinces (Sharif). It usually infests wild animals but may attack cattle, dog and goat.

In Mukteswar collection the species shows as its host the jackal.

*Haemaphysalis wellingtoni* Nuttall and Warburton.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 479-482; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 268.

Sharif gives the following distribution list:—

Gauhati (Assam) off a cock, and Andaman Islands.

*Haemaphysalis campanulata* Warburton.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 491-493; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 269.

The species has been recorded from Satharangapara (Travancore) and Kodarma (Bihar).

*Haemaphysalis choprai* Sharif.

Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 270.

The species is founded on a single female specimen off a wolf shot near Kodarma (Bihar).

*Haemaphysalis cornigera* Neumann.

Nuttall and Warburton [1915]. *Ticks*, Pt. 3, pp. 500-504; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 272.

Sharif records several specimens from Chittagong Hill Tracts off sambhar deer and dog. His record of the species from Kachugaon (Assam) also taken off sambhar, is from Mukteswar collection.

*H. cornigera* var. *anomala* Warburton has so far been recorded from Bihar and Orissa off cattle, buffalo and wolf (Sharif).

*Rhipicephalus sanguineus* (Latreille).

Latreille [1806]. *Genera Crustaceorum et Insectorum*, I, p. 157; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 279.

It is a chief parasite of dogs. Mukteswar collection shows the localities of infestation as follows :—

*Bengal*.—Calcutta off bullock.

*Assam*.—Gauhati off dog.

*Bihar*.—Purulia, Pusa, off dog.

*United Provinces*.—Mukteswar, Babugarh, Meerut, Bhim Tal, Kumaon Hills.

*Punjab*.—Rawalpindi off dogs ; Sialkot from human bedding.

*Kashmir*.—Jammu.

*Sind*.—Karachi off dog.

Sharif states that the species occasionally attacks cattle, horse, donkey, goat, wild boar, bear, fox, etc., and is widely distributed in India.

*Rhipicephalus haemaphysalooides* Supino.

Supino [1897]. *Atti. Soc. Veneto-Trent. Sci. Nat.* (2), III, p. 234 ; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 282-283.

This species as represented in Mukteswar collection has been recorded from the following places :—

*Bengal*.—Sahebganj off goat.

*United Provinces*.—Mukteswar in human habitation and off Pine Marten ; Babugarh off sheep.

*Sind*.—Off horse.

*Bombay*.—Off cattle ; Mahabaleswar off dog.

*Berar*.—Off cattle.

Of these records Sahebganj in Bengal, and Sind and Berar are new records to the long list already appended by Sharif. This species is of widespread occurrence, and attacks both wild and domestic animals.

*Boophilus australis* (Fuller).

Fuller [1899]. *Queensland Agri. J.* IV, p. 392 ; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 289.

The species is a major pest of cattle in India, and is represented in Mukteswar collection from the following localities :—

*Bengal*.—Off cattle.

*Assam*.—Bhowanipur off heifer ; Shillong, Gauhati and Mijiligaon off cattle.

*United Provinces*.—Mukteswar off hill bulls and nest of sparrow.

*Bihar*.—Bankipur off cattle.

*Central Provinces*.—Jubbulpore off cattle.

*Punjab*.—Murree off cattle; Rawalpindi off bullock.

*Central India*.—Mhow off cattle.

*Kashmir*.—Jammu off cattle.

*Bombay*.—Off cattle; Salkode and Nilkote off buffaloes.

*Hyderabad*.—Sitarampet off cattle.

*Berar*.—Off cattle.

Amongst these records Hyderabad, Berar and Kashmir appear to be new additions to the existing list already furnished by Sharif. The species has been recorded before from buffaloes.

*Boophilus annulatus* subsp. *calcaratus* (Birula).

Neumann [1911]. *Das Tierreich*, XXVI, p. 48; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 290.

The species is represented by one lot only in Mukteswar collection which was taken off cattle in Mysore. In addition to this Sharif records the species from cattle and horse in Coorg.

*Dermacentor auratus* Supino.

Supino [1897]. *Atti. Soc. Veneto-Trent. Sci. Nat.* (2), III, p. 235; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 296-297.

In Mukteswar collection this species is represented by one lot of specimens from Gonda (U. P.) taken off *Melursus ursinus*.

It normally attacks wild animals, and Sharif has given a detailed list of the localities including Assam, Bengal, Orissa, United Provinces and Bombay from where the species has been recorded so far.

*Nosomma monstrosum* (Nuttall and Warburton).

Nuttall and Warburton [1908]. *Proc. Cambridge Phil. Soc.* XIV, pp. 414-416; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 300.

The collection showing this species at Mukteswar is from Mahabaleswar (Bombay) taken off a dog in 1936. The species has previously been recorded from Bengal (Chittagong; parts of Midnapore District), Bihar and Orissa as also from Bombay (but in the latter Presidency from an unknown host). Dogs, cattle, buffalo, horse, sambhar and bear have been known to act as hosts.

*Hyalomma aegyptium* (Linn.).

Linnaeus [1758]. *Systema Naturae*, 10th ed., p. 615; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 305-306.

The author has observed this species in Mukteswar collection from the following localities :—

*United Provinces*.—Mukteswar, Allahabad, Babugarh off bulls, again from the latter place off horse and fowl; Cawnpore off cattle; Bhadri and Etah off goats.

*Punjab*.—Lahore off cattle.

*Bombay*.—Kumbheri, Bombay off cattle; Benkathi off sheep; Bombay off buffalo, goat and horse.

*Central India*.—Mhow off cattle.

*Bihar*.—Bankipore, Gaya off cattle.

*Central Provinces*.—Raipur off cattle.

*Hyderabad*.—Sitarampet off cattle.

*North-West Frontier Province*.—Peshawar off cattle.

*Berar*.—Off cattle.

*Baroda*.—Off cattle and horse.

*Sind*.—Karachi off goat and horse.

*Delhi*.—Off horse.

Along with *Boophilus australis* this species forms one of the serious pests of Indian cattle. This species has also been recorded from Madras by Sharif.

Of the three sub-species under *Hyalomma aegyptium*, viz., *dromedarii* Koch, *isaaci* Sharif and *ferozedini* Sharif, only *isaaci* has been represented in Mukteswar collection which was from Babugarh in Meerut District (U. P.). the host being not recorded.

*Hyalomma (Hyalommata) hussaini* Sharif.

Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 317-318.

This species along with its variety *brevipunctata* Sharif has been described by Sharif, and a long list of its distribution in the country given by the author ranges in Bihar, Orissa, Central Provinces, Madras and Bombay. The species has been mostly recorded from domestic animals such as cattle, buffalo, dog, goat, horse, etc.

*Hyalomma (Hyalommata) kumari* Sharif.

Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 320.

This species is represented in Mukteswar collection by a specimen from Sialkot (Punjab) which was found on a saddle lying for three years. Sharif records this species from Faridkot State (Punjab) off goat.

Other localities on record are variously distributed in Assam, Bihar, Orissa, United Provinces and Central Provinces (Sharif).

*Amblyomma integrum* Karsch.

Robinson [1926]. *Ticks*, II, Pt. 4, pp. 111-114; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 324-325.

This species has so far been recorded from Orissa, Bombay and Madras and in Muktiswar collection evidences of the species being collected from pig and buffalo exist. Its chief hosts appear to be cattle and buffalo.

*Amblyomma supinoi* Neumann.

Robinson [1926]. *Ticks*, II, Pt. 4, pp. 183-186; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 325.

The species has been recorded only from Baradighi in Jalpaiguri District (Bengal) off tortoise.

*Amblyomma sublaeve* Neumann.

Robinson [1926]. *Ticks*, II, Pt. 4, pp. 244-247; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 330.

The distribution of the species is known from certain localities in Bengal, Central Provinces, Punjab and Bombay and the species is especially restricted to Manis, Nicoria and Vesperugo.

*Amblyomma testudinarium* Koch.

Robinson [1926]. *Ticks*, II, Pt. 4, pp. 253-257; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 332-333.

The author has encountered the species in the collection at his disposal from Assam off Nellore cow and from Kalain (Assam) off tiger. It thus infests both domesticated and wild animals. Besides several places in Assam, the species has been known to infest various localities of Bengal, as also Kanara District of Bombay and Coorg State (Sharif). Bear, buffalo and goat are also probably attacked.

*Amblyomma hebraeum* Koch.

Robinson [1926]. *Ticks*, II, Pt. 4, pp. 104-107.

This appears to be an entirely new record as far as the literature at the author's disposal shows, and has been collected from Kalain (Assam) off tiger.

*Aponomma gervaisi* (Lucas).

Neumann [1899]. *Mém. Soc. Zool. France*, XII, pp. 182-185; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 337.

Sharif has recorded the presence of the species throughout the whole of India, its chief host being Varanus; Naia and Zamenis snakes are also frequently attacked. The species thus is not of economic importance.

*Aponomma gervaisi* var. *lucasi* Warburton.

Warburton [1910]. *Parasitol.* III, pp. 406-407; Sharif [1928]. *Rec. Ind. Mus.* XXX, pp. 339-340.

This species is widely distributed in several provinces of India and normally attacks the different snakes such as *Zamenis mucosus*, as shown in Mukteswar collection (this being obtained from Calcutta), Naia, Python, Vipera, Bangarus, etc. It also attacks Varanus.

*Aponomma laeve* Neumann.

Neumann [1899]. *Mém. Soc. Zool. France*, XII, pp. 190-191; Sharif [1928]. *Rec. Ind. Mus.* XXX, p. 341.

This species has been taken off cobra and rat snakes, like the other members of the genus, and recorded from Mahabaleswar (Bombay) and Coimbatore (Madras).

HOST-PARASITE LIST

Hosts

Man (*Homo sapiens*).

Parasites

- Ixodes acutitarsus.*
- \**Haemaphysalis flava.*
- H. aculeata.*
- Dermacentor auratus.*
- Rhipicephalus haemaphysaloides.*
- \**Ornithodoros papillipes.*
- O. savignyi.*
- Ixodes acutitarsus.*
- \**Haemaphysalis flava.* ]
- \**H. bispinosa.*
- \**H. bispinosa intermedia.*
- \**H. parva.*
- H. montgomeryi.*
- H. turturus.*
- H. spinigera.*
- H. leachi indica.*
- H. cornigera anomala.*
- \**Rhipicephalus sanguineus.*
- \**R. haemaphysaloides.*
- \**Boophilus australis.*
- \**Boophilus annulatus calcitratus.*
- Nosomma monstrosum.*
- \**Hyalomma aegyptium.*
- H. aegyptium isaci.*
- H. aegyptium ferozedini.*
- H. (Hyalommata) hussaini.*
- H. hussaini brevipunctata.*
- H. (Hyalommata) kumari.*
- \**Amblyomma testudinarium.*
- A. integrum.*

| Hosts                            | Parasites  |
|----------------------------------|--|
| Buffalo ( <i>Bos bubalis</i> ).  | * <i>Haemaphysalis bispinosa</i> .<br><i>H. spinigera</i> .<br><i>H. cornigera anomala</i> .<br><i>Rhipicephalus haemaphysaloides</i> .<br>* <i>Boophilus australis</i> .<br><i>Nosomma monstrosum</i> .<br>* <i>Hyalomma aegyptium</i> .<br><i>H. aegyptium issaci</i> .<br><i>H. aegyptium ferozedini</i> .<br><i>H. (Hyalommata) hussaini</i> .<br><i>H. (Hyalommata) kumari</i> .<br>* <i>Amblyomma integrum</i> . |
| Sheep ( <i>Ovis aries</i> ).     | * <i>Ornithodoros lahorensis</i> .<br><i>Ixodes ricinus</i> .<br>* <i>Haemaphysalis bispinosa</i> .<br><i>H. Sundrai</i> .<br><i>H. montgomeryi</i> .<br>* <i>Rhipicephalus haemaphysaloides</i> .<br><i>Boophilus australis</i> .<br>* <i>Hyalomma aegyptium</i> .<br><i>H. aegyptium issaci</i> .<br><i>H. (Hyalommata) kumari</i> .   |
| Goat ( <i>Capra hircus</i> ).    | * <i>Haemaphysalis bispinosa</i> .<br><i>H. swelli</i> .<br><i>Rhipicephalus sanguineus</i> .<br>* <i>R. haemaphysaloides</i> .<br><i>Boophilus australis</i> .<br>* <i>Hyalomma aegyptium</i> .<br><i>H. aegyptium issaci</i> .<br><i>H. (Hyalommata) hussaini</i> .<br><i>H. hussaini brevipunctata</i> .<br><i>H. (Hyalommata) kumari</i> .<br>* <i>Amblyomma</i> sp.   |
| Horse ( <i>Equus caballus</i> ). | * <i>Ornithodoros megnini</i> .<br>* <i>Haemaphysalis bispinosa</i> .<br>* <i>Rhipicephalus haemaphysaloides</i> .<br><i>R. sanguineus</i> .<br><i>Boophilus australis</i> .<br><i>B. annulatus calcaratus</i> .   |

| Hosts                                      | Parasites   |
|--|---|
| Horse ( <i>Equus caballus</i> )—contd.     | <i>Nosomma monstrosum</i> .<br>* <i>Hyalomma aegyptium</i> .<br><i>H. aegyptium isaaci</i> .<br><i>H. aegyptium ferozodini</i> .<br>* <i>H. (Hyalommata) kumari</i> .   |
| Donkey ( <i>Equus asinus</i> ).            | <i>Haemaphysalis bispinosa</i> .<br><i>Rhipicephalus sanguineus</i> .<br><i>R. haemaphysaloides</i> .<br><i>Hyalomma aegyptium</i> .  |
| Camel ( <i>Camelus dromedarius</i> ).      | * <i>Ornithodoros savignyi</i> .<br><i>Rhipicephalus haemaphysaloides</i> .<br><i>Boophilus australis</i> .<br><i>Hyalomma aegyptium</i> .<br><i>H. aegyptium dromedarii</i> .<br><i>H. aegyptium isaaci</i> .<br><i>H. (Hyalommata) hussaini</i> . |
| Barking deer ( <i>Cervulus unicolor</i> ). | * <i>Ixodes japonensis</i> .<br><i>Haemaphysalis bispinosa</i> .  |
| Sambhar deer ( <i>Cervus unicolor</i> ).   | * <i>Haemaphysalis cornigera</i> .<br><i>Nosomma monstrosum</i> .<br><i>Hyalomma hussaini brevipunctata</i> .<br><i>Amblyomma testudinarium</i> .   |
| Other deer.                                | <i>Haemaphysalis bispinosa</i> .<br><i>H. birmaniae</i> .<br><i>Rhipicephalus haemaphysaloides</i> .<br><i>Dermacentor auratus</i> .  |
| Bison.                                     | <i>Haemaphysalis bispinosa</i> .  |
| Pig ( <i>Sus domesticus</i> ).             | * <i>Amblyomma integrum</i> .   |
| Wild boar ( <i>Sus cristatus</i> ).        | <i>Rhipicephalus sanguineus</i> .<br><i>R. haemaphysaloides</i> .<br><i>Dermacentor auratus</i> .<br><i>Nosomma monstrosum</i> .  |
| Dog ( <i>Canis familiaris</i> ).           | * <i>Argas persicus</i> .<br>* <i>Ornithodoros papillipes</i> .<br><i>Ixodes ricinus</i> .<br>* <i>Haemaphysalis flava</i> .<br>* <i>H. bispinosa</i> .   |

| Hosts                                   | Parasites   |
|---|---|
| Dog ( <i>Canis familiaris</i> )—contd.  | <i>H. bispinosa intermedia.</i><br><i>H. leachi.</i><br><i>H. cornigera.</i><br><i>H. cornigera anomala.</i><br>* <i>Rhipicephalus sanguineus.</i><br>* <i>R. haemaphysaloides.</i><br>* <i>Nosomma monstrosum.</i><br><i>Hyalomma aegyptium.</i><br><i>H. aegyptium isaaci.</i><br><i>H. (Hyalommina) hussaini.</i><br><i>H. hussaini brevipunctata.</i><br><i>H. (Hyalommina) kumari.</i> |
| Cat ( <i>Felis domesticus</i> ).        | <i>Haemaphysalis bispinosa.</i>   |
| Tiger ( <i>Felis tigris</i> ).          | <i>Haemaphysalis hystricis.</i><br><i>H. bispinosa.</i><br><i>Rhipicephalus haemaphysaloides.</i><br><i>Hyalomma hussaini brevipunctata.</i><br><i>Hyalomma (Hyalommina) kumari.</i><br>* <i>Amblyomma testudinarium.</i><br>* <i>A. hebraicum.</i>   |
| Fishing cat ( <i>Felis viverrina</i> ). | <i>A. integrum.</i>   |
| Leopard ( <i>Felis pardus</i> ).        | <i>Haemaphysalis montgomeryi.</i><br><i>H. leachi.</i><br><i>Rhipicephalus haemaphysaloides.</i><br><i>Dermacentor auratus.</i>   |
| Bear ( <i>Melursus ursinus</i> ).       | <i>Haemaphysalis bispinosa intermedia.</i><br><i>Rhipicephalus sanguineus.</i><br><i>R. haemaphysaloides.</i><br>* <i>Dermacentor auratus.</i><br><i>Nosomma monstrosum.</i><br><i>Hyalomma aegyptium.</i><br><i>H. (Hyalommina) hussaini.</i><br>* <i>Amblyomma</i> sp.  |
| Wolf.                                   | <i>Haemaphysalis leachi.</i><br><i>H. campanula.</i><br><i>H. chuprai.</i><br><i>H. cornigera anomala.</i><br><i>Rhipicephalus haemaphysaloides.</i>  |

| Hosts   | Parasites  |
|---|--|
| Fox ( <i>Vulpes vulpes</i> ).   | * <i>Haemaphysalis flava</i> .<br><i>Rhipicephalus sanguineus</i> .<br><i>R. haemaphysaloides</i> .                  |
| Jackal ( <i>Canis aureus</i> ).   | * <i>Haemaphysalis leachii indica</i> .<br>* <i>Rhipicephalus haemaphysaloides</i> .<br><i>R. haemaphysaloides</i> . |
| Pine Marten ( <i>Martes martes</i> ).                                     | * <i>Rhipicephalus haemaphysaloides</i> .  |
| Sloth ( <i>Manis pentadactyla</i> ).                                      | <i>Amblyomma sublaeve</i> .  |
| Rabbit ( <i>Oryctolagus cuniculus</i> ).                                  | <i>Haemaphysalis bispinosa</i> .   |
| Hare.   | <i>Rhipicephalus haemaphysaloides</i> .  |
| Squirrel.   | <i>Ixodes granulatus</i> .   |
| Hedge hog.  | <i>Hyalomma aegyptium</i> .  |
| Fowl ( <i>Gallus gallus</i> ).  | * <i>Argas persicus</i> .<br><i>Haemaphysalis wellingtoni</i> .<br>* <i>Hyalomma aegyptium</i> .                     |
| Pheasant.   | * <i>Argas persicus</i> .  |
| Snakes (Cobra, rat snake, python) and<br>reptiles (Varanus, Calotes).     | <i>Aponomma gervaisi</i> .<br>* <i>A. gervaisi lucasi</i> .<br><i>A. laeve</i> .                                     |
| Tortoise ( <i>Testudo spp.</i> ).<br><br>( <i>Nicotria tricarinata</i> ). | <i>Hyalomma syriacum</i> .<br><i>Amblyomma clypeolatum</i> .<br><i>A. supinoi</i> .<br><br><i>A. sublaeve</i> .      |



## SELECTED ARTICLE

### SCHISTOSOMES AND SCHISTOSOMIASIS IN INDIA

BY

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ALTHOUGH fortunately schistosomiasis has not much significance in regard to the welfare of mankind in India, it has assumed paramount importance in connection with the health of domestic animals in this country. Compared with any other single group of parasites, schistosomes rank foremost in undermining the health of various of our domestic animals, and although the exact data in this connection are not available it will not be an exaggeration to say that they cause much inconvenience and harm to livestock in India, and inflict considerable monetary loss upon stock-owners in this country. In spite of this enormous loss, no thorough attempt has yet been made to bring together in a comprehensive manner all the maladies of domestic animals in which schistosomes are incriminated. We cannot, however, deny credit in this direction to Montgomery who, working at this Institute, published as early as 1906 two very comprehensive papers dealing with schistosomiasis of equines, cattle and sheep and discovered three new species of schistosomes. Since this pioneer work of Montgomery, there have appeared in this country, particularly in the last decade and a half, several valuable papers dealing with this group of parasites in relation either to domestic animals or human beings, and the present opportunity is, therefore, availed of to give a short résumé of all the work done in this country on this economically highly important group of parasites.

The earliest record of the occurrence of schistosomes in India was made by Cobbold, who in 1882, at a meeting of the Medical Chirurgical Society in London, declared that cattle and sheep in India are infected with schistosomes. Four years after this statement, Bomford reported the occurrence of *Schistosoma haematobium* eggs in the large intestine of two bullocks of the Transport Department destroyed in Calcutta. The dimensions of the egg were 0.17 mm.  $\times$  0.08 mm. *S. haematobium* has never been since recorded from cattle in this country and for this reason it is thought that the eggs

which Bomford observed in 1886 were those of *Schistosoma indicum*. The dimensions of the egg also corroborate this suggestion. This was followed by a long interval of two decades which was noteworthy for the absence of any records of schistosomes of domestic animals in this country. In 1906 appeared two memorable contributions from this Institute by Montgomery who described *Orinthalibharzia bomfordi* and *Schistosoma spindalis* from the portal circulation of cattle and *S. indicum* from that of the horse, donkey and sheep. Montgomery described all the symptoms and the pathological changes brought about by these worms in their respective hosts. He also recorded *S. bovis* from sheep in Bareilly and Lahore districts. He was of the opinion that *S. bovis* set up no pathological changes in sheep, but in the infection of sheep with *S. indicum* the alimentary canal from the pylorus to the margin of the anus is studded with small areas of distinct punctiform haemorrhages. Our recent observations at this Institute in the latter respect are in keeping with those of Montgomery. In some areas in this country, particularly Sind, Punjab, Assam and Bihar, there exists a condition of ovines known locally as *gillar* or *phet* characterised by persistent diarrhoea and oedematous swelling about the face. Examination of portions of intestine of sheep suffering from this disease received from Karachi revealed that the entire intestinal canal was studded with nodules which contained *Schistosoma indicum* worms and their ova. Baldrey's observations in 1906 at Lahore also corroborated this fact.

It has already been remarked that Montgomery described *S. indicum* and *S. spindalis*, and Bhalerao [1932] published more important data regarding the structure and the variations occurring in these worms. Liston and Soparkar in 1918 worked out the life-cycle of *S. spindalis* by artificially infecting a kid with the furcocerous cercaria liberated by the snail, *Indoplanorbis exustus* and by infecting snails with the miracidia hatched out of the spindle-shaped eggs obtained from a goat. Soparkar in 1921 published a detailed description of the cercaria of *S. spindalis*.

There exists in this country a peculiar snoring disease of cattle in which cauliflower-like growths are developed on the nasal septum. Malkani in 1932 published a short note on the etiology of this condition declaring that it is a form of nasal schistosomiasis. The same year Datta [1932] published a paper giving exhaustive details about the symptoms and the histopathology of the morbid tissue. In the succeeding year Rao and Malkani [1933] published their own observations on the symptomatology and the histopathology and other aspects of the disease. The question of the identity of the parasite was taken up by three workers in India : Bhalerao, Malkani and Rao. On the ground that the species *S. spindalis* is subject to many variations Bhalerao regarded the nasal schistosoma as a mere variety of *S. spindalis*, but he, being averse to the principle of multiplication of varieties within the range of the same species, abstained from assigning it a new variety name. Malkani, however, accomplished this and designated the nasal schistosome as *S.*

† This term is used merely for the sake of convenience.

*spindalis* var. *nasalis*. Rao went a step further and regards the nasal schistosome as a new species *S. nasalis*. It may be remarked here that the writers in the west such as Möning and Pillers uphold Bhalerao's view in this connection. One of the contentions in this respect is that it has been alleged that the nasal schistosome has been confined to the nasal area, but our recent knowledge on the subject does not substantiate this statement. A case from Assam revealed a typical Napoleon's hat-shaped ovum in a section of the intestine of a bull. Two more cases have been reported to the writer by a reliable worker in which the characteristic ova were found in the faeces of the infected animals.

Researches of Rao and Sewell have shown that the intermediate hosts of this nasal parasite have been *Limnaea lutelosa*, *L. acuminata* and *Indoplanorbis exustus*. Two important larval stages of this parasite, viz., miracidia and cercaria have been described by Rao and the disease has been artificially produced by him in cattle. The disease is also known to occur, although very rarely in buffaloes and goats.

Datta [1933] published a paper describing the nodular portal cirrhosis in enlarged liver of horses in India, causing persistent debility and heavy mortality as due to ova of *S. indicum*. He published a report of eight cases, described the morbid anatomy of the various organs of the horse and discussed the methods of diagnosis and control of the disease.

There exists in India a disease of equines, known locally as *kumri*, which resembles to a large extent the better known condition of equine paraplegia. Malkani in 1933 reported the occurrence of *Schistosoma indicum* worms from the liver and portal vessels of two *kumri* ponies at Patna. He obtained encouraging results by treating the *kumri* ponies with sodium antimony tartrate. On the basis of these two facts *S. indicum* has been looked upon by some as causing *kumri* in horses. This fact, however, requires corroboration before it can finally be accepted, for cases are known to the writer in which schistosomes were found but the animals did not show any symptoms of *kumri*.

Besides the animals referred to previously, camels also are infected with *S. indicum*. Record of this infection has been made by Leese [1911] who obtained the worms from the mesenteric veins of a camel in the Punjab.

Next in importance to the infection of domestic ruminants and equines with schistosomes comes the infection of pigs with these worms. Sewell [1919] recorded the occurrence of a cercaria resembling that of *S. japonicum* in *Indoplanorbis exustus* and *Limnaea amygdalum* in Calcutta. Bhalerao [1934] described male worms from the intestine of pigs in Calcutta which he regarded as a variety of *S. japonicum*, but since he is against the principle of the multiplicity of new variety names he preferred to designate the worms from the pigs in Calcutta as *S. japonicum*. The main difference between this

variety and the typical *S. japonicum* is that in the former the cuticle is tuberculate while in the latter it is smooth. From his previous studies on *S. spindalis* and *Ornithobilharzia turkestanicum* and of the specimens from pigs in Calcutta, he concludes that the nature of the cuticle is not of the specific significance.

Rao and Ayyar in 1933 described a new schistosome from the portal veins of pigs in Madras which they designated as *S. suis*. These authors consider that the eggs of *S. suis* and those that were obtained by Chandler in 1926 in human faeces are identical. If this be a fact, the specific name *S. suis* [Rao & Ayyar, 1933] falls into the synonymy of *S. incognitum* [Chandler, 1926] and not vice versa as imagined by Rao and Ayyar.

Very recently, Rao and Ayyar [1935] have recorded *S. incognitum* Chandler [Syn. *S. suis* Rao and Ayyar] from the faeces of a dog suffering from periodical dysentery, which was brought from Jubbulpore (Central Provinces) to Ootacamund (Nilgiris) for treatment.

There are very few records in this country of the indigenous infection of man with schistosomes. Powell [1903] reported a case of a native of Bombay who had never left the Bombay Presidency. Sewell [1904] recorded a case of *Schistosoma haematobium* occurring in a private of the 1st South Wales Borderer's. The patient had been four years in India and had never been to Egypt or South Africa. Christophers and Stephens [1905] mention a case of schistosome infection in a Madras native who passed eggs similar to those of *S. bovis* but somewhat larger and also eggs said to be indistinguishable from those of *S. haematobium*. Montgomery [1906] remarks that human schistosomes can live in this country. Chandler [1926] recorded the presence of schistosome ova with a subterminal spine in the faeces of a man from Bengal. Apart from these few records there has been no authentic proof of the existence of schistosome infection in man in this country. Several workers, however, have published from time to time, a record of schistosomiasis among people, particularly soldiers or pilgrims who had visited or stayed in areas of endemic infection : such workers being Hatch [1887], Powell [1903], Wardrop [1906], Crosthwait [1907], Milton [1914], Curjel [1918], Surveyor [1919], Franca and Mello [1921], Harkness [1922] and Cullen [1924].

Milton in 1919 put forward the view that human schistosomiasis is in all probability endemic in India and yet has hitherto entirely escaped the notice of medical men practising in this country. This statement of Milton created some stir among the scientists in this country and as an outcome of that there appeared in the same year three papers on the possible spread of schistosomiasis in this country. Sewell [1919] contradicts Milton and thinks that endemic schistosomiasis is not possible in India since the proper kind of molluscs do not exist in this country. Kemp and Gravely [1919] hold a view similar to that of Sewell and further record their failure to infect snails with the miracidia of human schistosomes in the neighbourhood of Hyderabad (Deccan) and Secunderabad. Soparkar in 1919 after discussing the pros and cons of the question concludes with the remarks that the question whether human

bilharziosis is likely to spread in India cannot, in the present state of our knowledge, be answered with any degree of certainty either in the affirmative or in the negative.

Besides the cases of natural infection dealt with so far, Fairley in 1927 and Fairley, Mackie and Jasudasan in 1930 tried to infect monkeys and guinea-pigs artificially with the cercariae of *Schistosoma spindalis* obtained from *Indoplanorbis exustus* and as a result of this they discovered that monkeys possess some natural immunity by virtue of which the development of cercaria does not proceed beyond the schistosomula stage in them. In the case of guinea-pigs they found that only the male worms attain full development while no female worms are demonstrable at autopsy. They suggest that in these animals some host factor antagonistic to the development of female schistosomes underlies the phenomenon of exclusive male survival rather than initial unisexual infestation. Two water-buffaloes were also successfully infected with *S. spindalis* by Fairley and Jasudasan [1927] and these buffaloes developed Bilharzial pseudotubercles in the liver within three months.

Coming now to the work on larval forms, besides the references to the work of Soparkar on the cercariae of *Schistosoma spindalis* and to that of Rao on the miracidia and cercariae of the nasal schistosome, Soparkar [1921] described from various molluscs in Bombay four new species of *Cercaria bombayensis* and Sewell [1922] recorded four new species of apharyngeal furcocercous cercariae. Sewell [1930] published a very valuable paper tracing the evolution of the excretory system in certain groups of furcocercous cercariae. As a result of a very exhaustive study, Sewell concludes that throughout both pharyngeal and apharyngeal forms there is a marked tendency for the more posterior flame-cells to undergo division before those that are situated more anteriorly, and in bifurcate cercariae there is a tendency for the anterior daughter cells produced by the division of the flame-cell in the tail stem to migrate into the distome body. In the same paper he also remarks that the suppression of the acetabulum and the consequent production of a monostome form has occurred on more than one occasion and on different lines of evolution.

Besides the successful use of antimesan and sodium antimony tartrate as anthelmintics in cases of nasal schistosomiasis and kumri, Fairley [1924] used emetine hydrochloride and tartar emetic in cases of *S. spindalis* infection of goats and he found that emetine hydrochloride possessed a maximal efficacy in the treatment of this species. In the same publication Fairley made a statement that this may prove to be the case in human bilharziosis as well. Christopherson [1925] remarks that Fairley is unjustified in considering that because emetine proved superior to antimony tartrate against *S. spindalis* in goats it will prove equally efficacious in human bilharziosis as well. He points out that in Fairley's own cases, emetine proved more toxic than antimony tartrate, and this is true for man also, as has been demonstrated by Kasr-el-Ainy in 1923.

There is yet another aspect of the subject which has not been dealt with so far and this is in connection with the work on metazoon immunity. The workers who deserve credit for having tackled this important branch of science are Fairley and his collaborators : Fairley in 1925 and 1926 performed several tests with the antigen derived from the dried powdered livers of *Indoplanorbis exustus* infected with the cercaria of *Schistosoma spindalis* and as a result of these tests he has drawn the following conclusions :—

- (a) The antigen which reacts with specific antibody in the complement fixation reaction for *Bilharzia* is a lipiod or lipoidal complex of colloidal nature.
- (b) The cercarial alcoholic extracts derived from the infected livers of *Indoplanorbis exustus* is strongly antigenic and will detect human schistosomes as well as *Schistosoma spindalis* and *S. indicum* in goats.
- (c) Intravenous injections of alcohol soluble extracts of cercaria cause a marked increase in the antibody content of the serum, but do not modify the course of the disease. Fairley and Jasudasan [1930] infected a large number of goats experimentally and numerous complement fixation tests were performed on each animal. Cercarial antigens of *Schistosoma spindalis* were used and the group nature of the cercarial antigen was proved for *S. haematum*, *S. mansoni*, *S. japonicum*, *S. bovis*, *S. spindalis*, and *S. indicum*.

Fairley and Jasudasan in 1930 published two papers :—one of them deals with the seasonal infection of the snails, *Indoplanorbis exustus*, with the cercaria of *S. spindalis* while the records of the experiments depicted in the other point to the conclusion that among ruminants alimentary infection with schistosomes is more important, since the contents of the rumen give an alkaline or neutral reaction. The schistosome cercaria cannot survive the normal acidity of the gastric contents in animals other than ruminants.

Finally, mention must be made of the work of Fairley and Mackie [1930] which deals with the pathological changes which the infection of *S. spindalis* brings about in the host tissues. They state that the lesions occurring prior to the deposition of ova include verminous phlebitis, involving the branches of mesenteric and portal veins, and toxic changes in the liver and kidneys pseudotubercles and periportal cellular infiltrations also occur in the liver. Hepatic pseudotubercles, verminous phlebitis of the main portal vein and its intrahepatic branches, mesenteric thrombosis and peripheral cirrhosis constitute the more common later manifestations. Although egg deposition occurs, macroscopical lesions in the small and large intestine are uncommon. Compared with other schistosomes the ova of *S. spindalis* are less toxic, but the worms produce more phlebitis. The liver invariably contains eggs and the deposition occurs in decreasing frequency in the large and small intestine, lungs, mesenteric glands, pancreas and other internal organs.

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## ABSTRACTS

The value of artificially dried grass, silage made with added molasses and A. I. V. fodder in the diet of the dairy cows and their effect on the quality of the milk, with special reference to the value of the non-protein nitrogen. WATSON, S. J. and FERGUSON, W. S. (*J. Agric. Sci.*, 26, 337-367, 1936).

An experiment was carried out to study the nutritive value of artificially dried grass and of silages prepared by two distinct processes, one with the addition of molasses which entails a fair degree of protein breakdown and the other A. I. V. silage where the protein breakdown is limited, in relation to the yield and quality of milk of dairy cows, with special reference to non-protein nitrogen whose feeding value has long been debated upon.

Four lots of four cows each were fed for a continuous period of 17 weeks, one lot on the control ration (ordinary winter ration) consisting of hay, roots and concentrates and the other three on artificially dried grass, molassed silage and A. I. V. fodder respectively which replaced a considerable part of the concentrates. The rations had been so adjusted that each supplied equal amounts of starch equivalent and digestible crude protein for maintenance and milk production. The experimental foodstuffs were all of good quality, high digestibility, with a high proportion of crude protein ranging from 17 to 21 per cent of the dry matter and supplying half of the total digestible crude protein in the ration. Artificially dried grass was fed at the rate of approximately 8 lb. and molassed silage and A. I. V. fodder at 30 lb. per head daily and these supplied 5·9 per cent, 31·4 per cent and 25 per cent of the total digestible crude protein intake in the form of non-protein nitrogenous compounds, besides the 10 per cent of the digestible crude protein in the form of non-protein nitrogen compounds supplied by the basal ration. The control ration was low in carotene whilst the experimental ones raised the carotene intake appreciably.

A statistical analysis of milk and butter-fat yields, butter-fat percentage and live-weight changes of the cows showed no significant differences between the four rations. The artificially dried grass diet had been the most efficient as regards its effects on solids-not-fat content of milk and the A. I. V. fodder the least. The other two rations showed no significant difference. The mean of all the cows showed a value just below that of the legal standard of 8·5 per cent solids-not-fat in milk and all the rations, except the artificially dried grass, were below the standard. The artificially dried grass, molassed silage and A. I. V. fodder had also increased the carotene content in milk and thus the vitamin A potency to a level equal to that of the grazing season for cows on pasture. Artificially dried grass, at the level fed, had replaced an equal weight of nutrients in the form of concentrated foodstuffs.

Thus for milk production the digestible crude protein of the foodstuffs, whether present in the form of non-protein compounds to the extent of 30-40 per cent of the nitrogen intake as in this experiment or as true protein, had all been utilized.

The nutritive value of non-protein nitrogen in these two silages proved high, at the levels fed, thus showing that digestible crude protein is a better measure of the value of the nitrogen in these feeds than either digestible true protein or protein equivalent values. [N. K.]

**Vitamin deficiency diseases in poultry.** BURMESTER, B. R. (*Vet. Med.*, 32, 8, p. 364).

With the development of poultry industry, involving as it does, intensification of production, has arisen the necessity for providing adequate nutrition, and in this connection the author gives a brief summary of the position regarding various avitaminooses in poultry.

Deficiency of Vitamin-K, a heat- and light-resistant, fat-soluble, unsaponifiable component of alfalfa or other green plants, produces a haemorrhagic condition in young chicks. The haemorrhages appear particularly in the muscle and in the subcutis, and are associated with an increase in the clotting time of the blood.

Chicks fed on diets to produce the above condition often show erosion of the gizzard lining. Experiments have proved that the latter is due to the deficiency of a fat-soluble, saponifiable component of green plants and cereals. The disease is very often noticed in chicks kept on common chick rations and can be prevented or cured by supplying large amounts of alfalfa leaf-meal.

The vitamin-B complex consists of several growth-promoting factors including the anti-neuritic Vitamin B<sub>1</sub> found in yeast, egg, liver, cereals and green feeds, and the anti-pellagra vitamin-G or B<sub>2</sub>. Vitamin-G has recently been split up into at least three components one of which is the yellow green pigment Flavin or Vitamin-G proper found in alfalfa, whey and egg white. Deficiency of Flavin produces impaired growth, emaciation, diarrhoea and death in chicks and dermatitis in turkey poult and rats. The other two factors are factor No. 1 (probably Vitamin B<sub>6</sub>) of unknown significance and factor No. 2, or "filtrate factor," which produces dermatitis in chicks but not in turkey poult or rats.

Other less-understood growth-promoting factors of the Vitamin-B Complex are vitamin B<sub>4</sub> and two others which, if excluded from the diet, produce defective bone-formation (micromelia) and encephalomalacia respectively.

Exclusion of Vitamin-A from the diet will produce nutritional roup and degenerative changes in the epithelium, thereby rendering the bird more susceptible to infection. Vitamin A is passed into the egg so that high-producing hens require a plentiful supply of it in their diet.

Deficiency of vitamin-D in the diet produces in the growing bird a type of leg weakness which recent investigations have shown to be analogous to osteoporosis and not to rickets. Chicks can utilize the vitamin-D of animal source better than that derived from plants. [M. Y. M.]

Ovine bacterial encephalitis (circling disease) and the bacterial genus *Listerella*. GILL, D. A. (*The Australian Veterinary Journal*, 13, 2, 46).

In his previous two communications to the Veterinary Journal in 1931 and 1933, the author had described an encephalomyelitis or "circling disease" in sheep in New Zealand, associated with a Gram-positive motile bacillus seen in the mid-brain, and had given the biological and cultural characters of the organism. In the present article he has placed the organism under the genus *Listerella* which has been only recently created [1927] but is likely to assume considerable importance in veterinary and medical science as it is known to cause fatal disease in rodents like rabbits and guinea-pigs, and in sheep, cattle, fowls and human beings. In support, a review of literature is given which would be particularly useful to workers interested in the subject.

Since the disease resembles louping ill but is distinct from it, Gill has proposed for it the name "Ovine Bacterial Encephalitis", and has placed the causative bacillus under the species *Listerella ovis*.

The disease appears to be widespread and has been reported from Britain, Australia and America. In New Zealand it occurs during the end of summer and early winter months and is responsible for heavy losses. The symptoms are indicative of encephalitis and include circling movement. The patient is, in a few days, unable to stand and dies quickly thereafter. At autopsy, apart from the congested meninges and cloudy cerebro-spinal fluid no other abnormality can be detected microscopically. Histologically, however, "cuffing" of blood vessels and foci of leucocytic infiltration, often containing the causative organism, are seen in the brain and spinal cord. The cerebral lesions are common in the mid-brain and the causative organism can be isolated from this site by inoculating blood agar plates with aseptically triturated material.

The cloudy cerebro-spinal fluid shows a greatly increased (over 2,000 cells per cubic mm.) cell count.

The ovine organism is rapidly fatal, by intravenous injection, to rabbits, the autopsy revealing acute meningitis and miliary necrotic foci in the liver. In both the situations the injected bacilli are abundant.

Broth cultures of the organism given subcutaneously (1 c.c.) into sheep had no effect; given daily (10 c.c.) by stomach tube, produced a rapid rise of temperature which returned to normal after the first three days; given intravenously (2 to 9 c.c.), produced a high fever, up to 108°F., which subsided after forty-eight hours; given into the carotid artery (1 c.c.), produced typical "circling disease" clinically and histologically; and given intramuscularly (10 c.c.), produced similarly positive results.

The natural mode of infection is believed to be via the respiratory tract, and in this connection larvae of *Oestrus ovis* are suspected.

The bacillus isolated by Gill grows with ease and equally satisfactorily under aerobic and anaerobic conditions. No exotoxin appears to be formed. A filtrable form of the organism could not be detected. In serum agar slope cultures the bacillus gradually tends to lose the Gram-positive character.

Work in connection with the epidemiology and immunology of the disease is indicated. [M. Y. M.]

**Viability of parasitic protozoa after the death of the host.** HEGNER, R. (*Am. J. Hyg.* 24, p. 309-31, 1936).

EXACT data on the period of viability of each type of protozoan organism in a dead host are not available in literature, but they are required for assisting in the diagnosis of specific infections, in the understanding of immunity reactions and also in the elaboration of effective public health measures. The author has, therefore, carried out experiments to determine the extent to which the customary belief that with the death of the host the harboured parasites die out, is justified. The usual difficulties in arriving at diagnosis due to partial or complete disintegration of parasites during the interim between death and autopsy, as for instance in fatal cases of human dysentery where it may not be possible to decide whether the original incitant of lesions happened to be *Entamoeba histolytica* or *Balantidium coli*, and also the possibility of the histological study of the host-parasite relationship being vitiated due to the migratory and penetrative action of parasites in dead tissues, as may happen in amoebiasis, have been mentioned. The mode of infection with human sarcocysts and with blood-inhabiting protozoa and the infectivity of parasites lodged in the host's tissues or when voided outside the animal body have been discussed. The contradictory results obtained in autopsical survey of intestinal protozoa of man carried out by previous workers have been mentioned.

Hegner's studies were restricted to protozoa in laboratory animals, including intestinal protozoa of rats, guinea pigs and frogs, malaria parasites in birds, and trypanosomes in rats. However, as the author points out, the experimental methods adopted by him are equally applicable in experiments with larger animals.

Faecal smears from small animals having been found to be positive for trichomonad flagellates, the animals were killed by cervical shock. The caecum was withdrawn through an abdominal slit, and portions of the caecal contents extracted at varying times. A faecal pellet culture method was adopted, and its result controlled by direct cover-slip preparations from the caecal contents. Each time a portion of the material was planted into 1 to 3 culture tubes, and the tubes were tested at the end of three days, and the caecal material was examined first every 3 hours and later at 24 hours' interval. A counting method was used in the case of Balantidial cysts. Trichomonad flagellates remained alive and multiplied on transference to suitable media, after being in the caecum of a dead rat at 37°C. for 24 hours, at room temperature for 4 days, and at 5°C. for 13 days. Balantidial trophozoites were viable in the caecum of dead guinea pigs at room temperature or refrigerator for at least 4 days. Pig Balantidium when kept in Ringer's solution were viable at room temperature for 6 days. Intestinal protozoa of frogs, *Nyctotherus*, *Opalina*, *Trichomonas* and *Hexamita* were found to live up to 20 days in dead frogs. The ciliates were apparently less resistant than flagellates. *Plasmodium cathemerium* in canaries is capable of infecting after the bird has been dead for 2 days, and *Trypanosoma lewisi* remained alive in the body of dead rats at room temperature for 3 days, and at 5°C. for 10 days. The parasites in a dead rat kept at 5°C. were capable of infecting clean rats after 9 days. Further the author attempted to demonstrate the failure of the trypanocidal antibody to appear at the peak of infection in dead rats. [S. C. A. D.]

## NOTE

### Far Eastern Association of Tropical Medicine

#### 10TH CONGRESS

THE Tenth Congress of the Far Eastern Association of Tropical Medicine will be held at Hanoi (Address "Igesante", Hanoi, Indochine) from the 24th to 30th November, 1938.

All licensed Medical, Dental and Veterinary practitioners are eligible for membership. The membership fee for the period 1934-38 is £3 (or Rs. 40-2-0) and should be paid to the Local Provincial Secretaries of the Far Eastern Association of Tropical Medicine, to whom the names of members in their areas should be submitted. The members are also requested to inform the Local Secretaries whether they propose attending the Congress. The titles of any papers which it is proposed to place before the Congress should be submitted to the Local Secretaries at an early date. Arrangements will be made for the reading at the Congress of any paper submitted by a member who is unable to attend.

The Ninth Congress held at Nanking in 1934 decided that sections on Food Problems and Sanitary Measures with reference to Sewage and Garbage Disposal should also be added to the programme of the Tenth Congress.

Further information may be obtained from the Local Provincial Secretaries or from Lt.-Col. G. Covell, M.D., D.P.H., D.T.M. & H., F.R.E.S., I.M.S., Director, Malaria Institute of India, and Local Secretary of the Far Eastern Association of Tropical Medicine for Government of India, Kasauli, Punjab, or the Honorary General Secretary, Far Eastern Association of Tropical Medicine, Parapattan 10, Batavia (Centrum) Java.



## CORRIGENDA

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*The Indian Journal of Veterinary Science and Animal Husbandry, Vol. VIII,  
Part I, (March, 1938).*

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Page 36, Table II, 5th, 6th and 8th columns—

*For '100' read '<100'.*

Page 37, Table III, 4th column—

*For '50' read '<50'.*

Page 38, Table IV, 6th column, last figure—

*For '25' read '<25'.*

Page 39, Table V, 3rd, 4th, 5th and 6th columns—

*For '50' read '<50'.*



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## ORIGINAL ARTICLES

### THE PROBLEM OF VITAMIN-A DEFICIENCY IN THE DIET OF FARM ANIMALS\*

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(Received for publication on 25th May 1938)

#### INTRODUCTION

THE discovery of the fact that the proper growth, health and reproduction of an animal involved other factors than a sufficiency of calories and protein has shifted the centre of interest in nutritional studies from quantity to quality. Among these accessory constituents of food, vitamin-A occupies a most important place. Although this substance has now become determinable by chemical and spectroscopic methods, our knowledge with regard to the requirements of the various animals and the conditions favourable to its proper utilization is so limited that the adequacy or inadequacy of a diet has to be judged by clinical observations only.

The symptoms characteristic of avitaminosis-A are more or less well-defined. It would, however, be interesting to notice some recent cases on record. Fed on over-ripe timothy hay as sole roughage in addition to a mixture containing yellow corn meal, linseed meal, soya bean meal and wheat bran for about twelve months, four cows, negative to contagious abortion, consistently failed to have normal calves [Converse and Meigs, 1931]. A ration for pigs made up of white corn, buckwheat middlings, tankage and minerals or white corn, oats, linseed oil meal, tankage and minerals resulted in serious eye troubles, paralysis, respiratory trouble and convulsions [Longwell and Weakley, Jr., 1932]. A sow on a vitamin-A deficient diet gave birth to a litter of eleven pigs, all without eyelids [Hale, 1932]. When the mother had received a diet short in vitamin-A content before mating and during the first thirty days of gestation, three litters of pigs were born with-

\* Paper read before the Section of Veterinary Research of the Indian Science Congress, held in Calcutta, January, 1938.

out eyeballs or with very serious eye defects [Hale, 1935]. It was shown that on a low vitamin-A diet, female rats were affected by a prolongation of the gestation period, with death of foetus in utero and failure of the mechanism of parturition, despite an abundant supply of vitamin-E [Mason, 1934, 1935]. Rabbits, guinea-pigs and albino rats developed xerophthalmia on a diet low in vitamin-A content while one monkey out of twenty-seven developed typical xerophthalmia with keratomalacia in both eyes [Hetler, 1934]. An adverse effect on the prostate was seen in eight pre-puberal rats aged thirty days and eight post-puberal ones aged 100 days all receiving a vitamin-A deficient diet. The change was taken to indicate a failure in the secretion of the male sex hormone. In five extreme cases of human malnutrition similar changes were also observed [Moore and Mark, 1936]. On a ration consisting of white corn ninety parts, fish meal seven and mineral mixture five, pigs, kept in sunlight but with no pasture, ceased to grow after 150 days and developed muscular in-co-ordination with spasms, lameness, lachrymation, blindness due to ulceration, inflammation and congestion of the intestinal tracts and finally succumbed. White corn in the diet introduced a vitamin-A deficiency, since rats fed on it developed ophthalmia, lost weight and died even when the diet was irradiated and supplemented with five per cent yeast. Rat experiments and bone analysis showed that the diet was sufficient in vitamins B<sub>1</sub>, B<sub>2</sub> and D [Hostetler *et al.*, 1935].

All the disorders mentioned above are both curable and preventable by an adequate supply of vitamin-A. The liver being the store house for this material in the animal body, the best sources of vitamin-A must necessarily be the livers themselves or their extracts properly concentrated. Escarole, cod-liver oil, halibut liver oil and "vogan" are some of such concentrates. While these may be important as therapeutic doses for curative purposes, to include them in the regular dietary of animals would not be a practical proposition. Various investigations have, therefore, been carried out to determine the suitability or otherwise of different vegetables and plants as vitamin-A supplements.

#### CAROTENE AS THE SOURCE OF VITAMIN-A IN PLANTS

It is generally known that while vitamin-A as such is not available in plants, all green leaved or yellow rooted vegetables or plants contain various quantities of carotene, the precursor of vitamin-A, particularly in the earlier stages of growth. It has also been shown in an investigation on fifty-nine plant carotenoids that while  $\beta$ -carotene was the main constituent in all cases,  $\alpha$ -carotene was also present in quantities varying from traces to about thirty-five per cent of the total carotene content [Mackinney, 1935]. The carotene in these plant sources is also convertible into vitamin-A in the animal system. It was noticed that while swine, sheep, goats and rats almost completely converted their carotene in the feed into the colourless vitamin-A before storage or secretion, cattle were capable of conserving and secreting their vitamin-A

both in the form of carotene and as colourless vitamin-A [Hart and Guillet, 1933]. In an experiment on cows it was observed that there were small amounts of carotene and much larger amounts of vitamin-A in the liver and milk fats. By feeding carrots, a rich source of carotene, at the end of winter feeding, both the vitamin-A and the carotene contents of butter increased [Moore, 1932]. It becomes clear, therefore, that so far as animals are concerned, the problem of vitamin-A deficiency resolves itself into an adequate provision of carotene in their normal feed which consists of grains, milled products and forage crops.

Many observations have been recorded to the effect that carotene is closely associated with the pigments in a material. Yellow maize was found to be richer in vitamin-A than white maize [Mangelsdorf and Fraps, 1931]. Yellow corn and yellow corn meal seemed to be rich sources of vitamin-A while white corn contained little, if any. It was also noticed that the soil and season had influences on the vitamin-A content [Fraps, 1931]. Flour from wheat grown on irrigated land had a lower carotene content than the flour from dry land wheat [Whiteside, 1931]. In five varieties of maize it was found that the yellow variety had a higher vitamin-A potency than the white ones. The correlation between vitamin-A content and colour was true only with respect to the endosperm, the depth of colour of the pericarp being immaterial [Takahashi and Masuda, 1935]. In studying the distribution of carotene in the wheat grain, it was also observed that the variety of wheat had a greater influence than location, season or storage. The flour prepared in each case had a lower carotene content than the whole wheat. Bran had the highest concentration of carotene, then middlings, second patent flour and first clear flour, while the first patent flour had the least [Fifield, 1936]. The carotene content of twenty-nine varieties of whole wheat and seventy-two hybrid strains showed variations in two years of 1.66—3.80 parts per million and 1.80—3.80 parts per million. This work also showed that the carotenoid pigmentation of wheat was an inheritable characteristic, some of the strains being homozygous and some heterozygous for this characteristic. The breeding of wheat of a given carotene content would thus appear to be possible [Worzella and Cutler, 1935].

Notwithstanding these studies on the carotene content of some cereals, grain feeding as such has not been considered as a source contributing to the vitamin-A needs of animals. White corn and oats as the main feed of kids resulted in serious avitaminosis-A [Longwell and Weakley, 1932]. To a vitamin-A free diet of young rats a supplement of two grm. daily of English wheat, oats or barley was added from the twentieth day. The animals grew well for about three weeks and then lost weight and died. The initial stimulus to growth was attributed to the xanthophyll in the cereals and not to any carotene therein [Malimberg and Euler, 1936]. A basal ration containing white corn, fish meal and mineral mixture caused a cessation of growth in pigs and later induced a severe type of avitaminosis-A [Hostetler *et al.*, 1935]. The vitamin-A potency due to the carotene in the butter of a cow fed on

white maize decreased from thirty per cent at the beginning of the experiment to one per cent near the end. With yellow maize instead, the decrease was from forty-seven to nine per cent only [Treischler *et al.*, 1935].

With regard to the soya bean too, the available data are conflicting. In fresh soya bean grown near Leipzig, vitamin-A was found in traces [Scheunert and Schieblich, 1935] while other experiments showed that the soya bean was so rich in vitamin-A that it could serve as the sole source of vitamin-A for feeding growing rats [Iwanova, 1935]. The carotene content of soya beans in India has been estimated as 710 international units per 100 grm. [Aykroyd, 1936]. On the other hand, when the linseed oil meal in the grain feed of a dairy ration was replaced by raw or cooked soya beans, the vitamin-A value of butter went down from the neighbourhood of 23 to 28 Sherman units to about 15 to 18 units per grm. The replacement of alfalfa hay by soya bean hay had also a similar effect [Wilbur *et al.*, 1935].

It should be mentioned here that vegetable oils such as earthnut, gingelly, cocoanut, linseed, mustard and hemp seed oils were found to contain very negligible traces of vitamin-A, red palm oil being the only exception [Rosedale and Oliviero, 1934; Lo T-Y, 1935]. A spectrographic study of several vegetable oils in this country also showed that they were all negative for vitamin-A [De, 1935]. Vitamin-A being a fat soluble one, the oil cakes that are generally used in animal feeding would not contain any significant amount of this constituent. It is apparent, therefore, that to provide an adequate supply of vitamin-A in the feed of animals in general and farm stock in particular, a proper choice of forage plants either as the main feed or as a supplement is the only possible method.

It would be useful in this connection to remember that there are certain factors which affect the vitamin-A content of vegetables and plants in general. White, yellow, orange, garden and field varieties of carrots, grown under greenhouse conditions, showed on analysis that the most highly coloured carrots contained 9.6 mg. of carotene per 100 grm. while white varieties yielded only 1/80th of this amount [Bills and McDonald, 1932]. The vitamin-A content of the outer green leaves of Iceberg lettuce was found to be 34.5 Sherman units per grm. of leaf, while the inner bleached leaves gave a content of 1 unit [Munsell and Kennedy, 1935]. A study of the variations in the carotene content of Kentucky blue grass, green growing alfalfa, carrot, etc., revealed the fact that green plants were very rich and dried or brown leaves very poor in carotene [Shinu *et al.*, 1935]. The carotene content of the corn plant and of the silage made from it was found to depend upon the greenness of the plant at cutting [Kane and Cary, 1935]. Veld grass samples from Bechuanaland showed a low carotene content at different seasons during two years. Only the fresh growth after rain was rich and there was progressive deterioration as the pasture dried up [Myburgh, 1936].

It has further been observed that manuring had no effect on the carotene or the vitamin-A activity of Cumbu leaves and that under all conditions of growing the carotene content decreased with the age of the crop (Sci. Rep.

Govt. Agric. Chem. Coimbatore, 1932-33). Vegetables had their carotene content markedly increased by a fertilization of the soil with nitrogen, phosphate and potash. Irradiation by neon light of parsley, chive and spinach increased their yield and vitamin-A content [Pfutzer and Pfaff, 1935].

Several attempts have been made to estimate the carotene content of some of the commoner pasture plants in their fresh green stage. Timothy grass contained  $200 \pm 13$  Sherman units per grm. of fresh material, red top  $308 \pm 10$ , smooth brome grass  $396 \pm 27$ , alfalfa  $269 \pm 17$ , white blossom sweet clover  $242 \pm 19$  and  $500 \pm 30$  respectively in the second and first years, orchard grass  $275 \pm 13$  and meadow fescu  $250 \pm 13$ . Based on these results it has been advised that dairymen could select any mixture of pasture plants adapted to their particular climatic and soil conditions without any risk of producing a vitamin-A deficiency [Woods, *et al.*, 1935, 1935-a, 1935-b]. In making an estimate of the vitamins in the diet of pigs, the vitamin-A contents of air-dried rye grass and sun-cured pasture hay have been put down as 3,500 and 1,000 international units per 100 grm. [Cunningham, 1936].

#### VITAMIN-A REQUIREMENT OF ANIMALS

We have so far concerned ourselves with avitaminosis-A and the possible sources through which it can be averted in so far as it relates to animal nutrition. The effect of providing the animal with its normal requirement of vitamin-A would just enable the animal to have its proper health, growth and production. It is, therefore, desirable to obtain an idea as to the requirements of the various animals for carotene or vitamin-A for prophylactic and curative purposes.

To prevent and cure xerophthalmia in rats on a vitamin-A deficient diet, it was found sufficient to give a daily dose of  $0.5$  to  $1.0$  γ of three different specimens of carotene. Other workers found that 2 to 5 γ were necessary [Polak and Stokvis, 1931]. As a result of studies on thirty-one puppies, depleted of vitamin-A by feeding, it was shown that 114 U. S. P. units per 100 grm. of body weight were required before any appreciable storage could occur [Frohring, 1935]. The requirements of the fowl per unit of body weight was observed to be more than that of the rat for growth and maintenance. Depleted birds of about 400 grm. weight required about 0.05 mg. of carotene daily, while fowls weighing 2,000 grm. had to be given 0.5 mg. For prophylactic purposes slightly smaller quantities might be sufficient. White Leghorns required thirty-three Sherman units per pound of live weight every day for maintenance and 6.3 units to produce one unit of vitamin-A in the egg yolk, the yolk in an average egg weighing fifteen grm., each grm. of which would contain twenty units of vitamin-A [Cruickshank, 1935].

Pigs on the other hand have been found to require only small quantities of vitamin-A. On a vitamin-A free diet they continued in normal health till the third litter. Pigs born at this stage failed to thrive on the same deficient diet. But young ones born to normally fed parents grew up in proper condition to the fattening stage, even on a vitamin-A free diet. Green fodder or

yellow maize was deemed a sufficient vitamin-A supplement, any further additions of vitamin-A concentrates to the ration being of no additional benefit [Sheehy, 1932]. It has, however, been pointed out that a 100 lb. pig should have the equivalent of four mg. carotene daily in its diet to satisfy its vitamin-A requirements for maintenance and normal growth [Dunlop, 1935].

In an investigation into the minimum vitamin-A requirements of cattle, it was noticed that animals receiving a small daily ration of cod-liver oil or alfalfa meal for two years showed scarcely any storage, the total reserve of the body being estimated at 200 mg. of carotene and vitamin-A. It was also determined that the rate of depletion of this reserve was about 9 to 11 γ per kg. of body weight per day. For animals depleted till night blindness had set in, the minimum daily dose to enable them to have a normal health and growth was found to be 26 to 33 γ per day per kg. of live weight [Guilbert and Hart, 1935]. On this basis the approximate requirement of a 1,000 lb. bullock worked out to be 11.7 to 14.85 mg. of pure β-carotene per day. Besides the above mentioned maintenance requirement, cows would have to secrete large amounts daily in their milk. The vitamin-A value of the best grade of cows' milk was found to be 1,500 rat units per quart. It was also observed that only less than five per cent of the carotene content of the food was transmitted through the milk [Russell, 1933]. In another experiment it was recorded that the cows' milk contained 2,500 to 1,600 international units per quart and that more than 3.5 per cent of the feed carotene was never present in the milk [Russell *et al.*, 1935]. A biological estimation of the vitamin-A content of whole milk showed it to be about 454 international units per pint [Cunningham, 1936]. When the carotene or vitamin-A ingestion was low, cows secreted 3.3 per cent of the intake through its milk and when on carotene rich feed 1.3 per cent only [Garry *et al.*, 1936].

#### STORAGE AND UTILIZATION OF VITAMIN-A

Having considered the vitamin-A requirements of animals for maintenance, growth and health, it is natural to suppose that the excess amount in the feed of the animals would be stored in the system for use in productive purposes. This takes us on to the question of the animals' capacity to store and the mechanism through which they eliminate this constituent.

It has been found that of the total amount fed to a horse about 70 to 80 per cent of the carotene and 55 to 64 per cent of the xanthophyll were excreted in the faeces. An examination of the dung pigments showed that they remained unchanged by the digestive process [Zechmeister and Tuzson, 1934]. Another experiment showed that the vitamin-A excess was mainly excreted in the urine as a degradation product, giving it an increased iodine value [Santos Ruis, 1935].

Quantities of vitamin-A in the feed which escaped elimination through either of these channels should have found their way into the store of the body, namely, the liver, which is also the seat of carotene metabolism. An

assay of the vitamin-A contents of the normal liver of some animals such as oxen, guinea-pigs, rabbits, rats and dogs showed considerable variations, the liver of the guinea-pig being the least and that of the oxen the most potent [Simmonet *et al.*, 1932]. Goat liver was found to have a reserve more than twice that of the bull liver and six times that of rabbit liver [Sen and Sharma, 1936]. The livers of cows had about four to five times the vitamin-A contents of those of bullocks, while calves showed no such difference between the sexes [Ender, 1934]. Eighteen-month-old rats which had finished breeding were fed for about twelve weeks on a vitamin-A sufficient diet superimposed by a large amount of some concentrate. The vitamin-A concentration in the liver at the end of this period was 18,000 blue units per grm. Put on to a deficient diet at this stage, the concentration fell down to 2,700 units per grm. in four weeks and in twelve weeks to about 400 units [Davies and Moore, 1935]. Some beef steers receiving throughout the summer a diet found optimal for storage purposes were changed to a diet deficient in vitamin-A. Sixty-three, 121 and 282 days later it was estimated that there was a progressive decrease in the vitamin-A of the livers showing that there was a continuous depletion from the amounts stored when they had been fed in excess. The concentration of vitamin-A in the livers of cows receiving a rich ration was found to be approximating to that in cod-liver oil. New born calves from well-fed cows showed only a low concentration of vitamin-A in their livers, although the colostrum was rich [Guilbert and Hart, 1934]. Rats which had received vitamin-A to the order of 5,000 times the necessary minimum before and during gestation threw young ones with only small amounts in their livers. These amounts could be slightly increased by feeding a diet to the mother which was rich in fat as well as in vitamin-A [Dann, 1934].

Although it has been shown that a high vitamin-A reserve in the liver of a cow did not give rise to a high vitamin-A content in the liver of her calf, it has been shown by several workers that it is immediately reflected in her milk and butter. The fact that the carotene and vitamin content of the feed exert a considerable and proportionate influence upon the carotene and vitamin-A of the milk and butter-fat has been borne out by a large volume of work wherein studies have been made with various concentrates and vegetable and plant feed as sources of the vitamin-A intake [Moore, 1932; Fraps and Treichler, 1932; Copeland and Fraps, 1932; Watson *et al.*, 1933; Gillam *et al.*, 1933; Baumann *et al.*, 1934; Fraps *et al.*, 1934; Converse *et al.*, 1935; Beeson, 1935; Hilton *et al.*, 1935; Wilbur *et al.*, 1935; Treichler, 1935; Steensberg, 1936].

During the course of the work on the effect of the feed on the carotene and vitamin-A content of milk and butter, it was observed that the breed of the animal was also a factor in transmitting the reserves in the system through the milk. In cows kept under comparable conditions, Guernsey cows' butter gave a carotene value of 7.8 γ per grm. while Holstein and Ayrshire gave 4.3 γ per grm. The vitamin-A content on the other hand was only 5.1 γ in Guernsey while it was 10.1 γ in the case of the Holstein.

Brown Swiss and Jersey cows gave intermediate values [Baumann *et al.*, 1934]. In a comparison between Holstein and Ayrshire butters it was found that biologically both had the same total vitamin-A potency [Beeson, 1935]. The butter-fats of Guernsey and Holstein breeds were approximately equal in vitamin-A potency but definitely superior to those of the Ayrshire and Jersey. The carotene contents under winter conditions were, Ayrshire 1.45 mg. per kg., Holstein 1.95, Jersey 2.05 and Guernsey 3.45; under summer pasture the figures were 4.7, 8.0, 12.1 and 20.5 mg. carotene per kg. of butter respectively [Sutton and Krauss, 1936]. Another experiment showed that the carotene and vitamin-A value of the butter of Guernsey, Friesian, Ayrshire and Shorthorn cows were in descending order [Gillam *et al.*, 1936]. It has been suggested that each breed of cow would have a "ceiling value" above which it would not be possible to increase the vitamin-A potency of its milk or butter [Gillam and Heilbron, 1934].

#### CONSERVATION OF VITAMIN-A POTENCY OF ROUGHAGES

It has already been found that most forage plants in their fresh young stage yield a sufficiency of vitamin-A to animals fed or grazed on them. If an animal is, therefore, assured of green feed as a major fraction of its roughage, then the vitamin-A problem in cattle ceases to exist. But, however, desirable it may be, it may not be possible under practical conditions of farming to provide such feed throughout the year. The problem, thus, reduces itself to a question of fodder preservation with a view to retain the carotene content of the plant at the optimum level.

Ensilage is one of the methods of preservation which has been tried in this connection. In studying the effects of the feed on the carotene content of butter, it was observed that forty lb. of A. I. V. silage in the feed raised the yellow colour to a level comparable with that found on best pasture, while the vitamin-A content was doubled [Gillam and Heilbron, 1932]. Corn silage was found to be a valuable source of vitamin-A for feeding dairy animals [Russell *et al.*, 1935]. A. I. V. silage made from grass and clover or young alfalfa were tested as suitable vitamin-A feeds to dairy cows. It was found that thirty kg. of the former and fourteen kg. of the latter gave adequate response [Steensberg, 1936]. Winter milk from cows fed on A. I. V. silage was as high in vitamin-A potency as that of summer milk on pasture, namely, about three times that of cows fed on hay, concentrates and roots. The A. I. V. process resulted in good silage which preserved the carotene and vitamin-A contents of the green plants intact. It may also be pointed out incidentally that while hay-making destroyed the vitamin-C entirely, in the A. I. V. silage half of it was preserved [Virtanen, 1935].

Hay-making is another method of fodder preservation which can be utilised for the preservation of the carotene content of fodder plants. The factors involved in hay-making are so prejudicial to the retention of carotene or vitamin-A activity that much attention has been devoted to finding out the best process of hay-making to suit the vitamin-A requirements of animals.

Studying the effects of ultra-violet radiations on vitamin-A, it was shown by biological tests that up to one hour of exposure there was no loss, while after four hours of irradiation only 1·5 per cent of the original content remained. But the colour test showed that the destruction started at once and that there still remained 30 per cent after thirty-two hours of exposure [Norris, 1931]. The vitamin-A content in alfalfa was found to decrease with age whether kept ground or chopped. Curing alfalfa in cocks in the field had the same effect as curing in diffuse light of varying intensity. When this hay was cured in windrows exposed to sunlight, only 2/3rds of the potency was lost while the hay exposed to sunlight with rain and dew lost 3/4ths [Hartman, 1931]. Drying alfalfa in the field caused a greater loss of potency, probably through enzyme action, than when dried by hot air or hot flue gas. Warmth and moisture favoured the destruction of vitamin-A, whilst cold, rapid drying or autoclaving hindered it [Hauge and Aitkenhead, 1931]. Alfalfa hay, protected from the sun while curing, contained four to five times as much vitamin-A as the hay exposed to the sun and rain [Reed, 1931]. Alfalfa which was removed from the field as soon as cut and stored in a dark, well-ventilated room, was compared with exposed ones. Exposure for 2½ to 6½ hours led to a loss of twenty to thirty per cent of vitamin-A but none of the green colour. Overnight exposure in the field caused a loss of seventy-five per cent of its content while a week's sun and rain bleached the hay severely and destroyed about ninety-six per cent of its potency [Smith and Briggs, 1933]. Sun-cured hay exposed to one inch or more of rain was less potent than that cured in diffuse sunlight, the vitamin-A content varying from 10 to 60 Sherman units per grm. of hay. Growth of moulds had deleterious effects on all vitamins [Douglas *et al.*, 1933]. The vitamin-A and -D contents of hay dried quickly at 68° C. in two hours were much greater than when slowly dried in meadows or frames [Rygh, 1934]. Machine dried alfalfa has a higher vitamin-A content than the field dried ones. Rapid loss of carotene occurred in field curing in day light. Dried alfalfa, finely ground, could be stored in vacuo at 0° C. without loss [Russell *et al.*, 1934]. Vacuum dried alfalfa, dried for three hours at 100° C., had as much carotene as the fresh leaves, while the field cured ones and the commercially dehydrated ones had the same content of carotene, 25·3 mg. per 100 grm. The vacuum dried alfalfa hay contained 52 mg. per 100 grm. [Guilbert, 1934]. Vitamin-A potency of freshly cut alfalfa could be retained if the enzymes were destroyed immediately before drying. The destruction attributed to sunlight might be attributable to the higher temperatures produced accelerating the enzymatic activity [Hauge, 1935]. No loss was noticed on vacuum drying but autoclaving and sun-drying caused considerable losses. In slow drying, enzymic and bacterial action had their part. Below 5° C. a storage of six months did not show any loss. Temperature was found to be the most influencing factor in decreasing the vitamin-A potency of hay [Guilbert, 1935]. When alfalfa was cut and cured in the cock in fresh condition it had  $\alpha$ -vitamin-A content of  $308 \pm 13$  units per grm. On being ground and stored for four months it was  $233 \pm 20$ . When exposed in the swathe for one day before cocking it had  $144 \pm 10$  units per grm., whereas

an exposure for three days before cooking gave a hay with only  $116 \pm 9$  units per grm. It was also found that cock cured and unstacked hay kept for two and a half months contained  $233 \pm 20$  units per grm. but hay stacked for forty-nine days had only  $144 \pm 10$  units [Woods *et al.*, 1936].

Briefly stated, the provision of an adequate supply of vitamin-A in the dietary of animals, particularly cattle can be accomplished only by making their natural feed richer in carotene content and not by feeding vitamin-A concentrates. In India their main feed consists generally of a roughage and a concentrate ration, the feeding of carrots and other roots not being common. The concentrate ration is generally composed of grains and oil-cakes which cannot be made to yield appreciable amounts of vitamin-A. Hence animals have to be provided with fresh green feeds, which under ordinary farm conditions provide an adequate amount of carotene. As it may not be possible to provide such a feed throughout the year on any large scale, the methods of preservation of fodder plants without destruction of the vitamin-A potency have to be investigated. Ensilage and hay-making are the two methods open and considerable work has to be done with regard to these aspects of the question. It should also be stated that by proper plant breeding, fodder crops with improved carotene contents could be developed.

#### CONCLUSION

In the foregoing pages, a general review has been given of the carotene contents of various stock feeds and the requirements of vitamin-A for the proper maintenance of the health of live-stock. It would be appropriate here to consider the question of avitaminosis-A under Indian conditions. It has been shown elsewhere [Guilbert *et al.*, 1934] that vitamin-A deficiency in the diet of farm stock may occur both under stall feeding conditions as well as when grazing and the common symptoms are—

- (a) expulsion of the foetus prematurely or dead at term or birth of weak calves with or without eye lesions and associated with retention of the placenta,
- (b) severe diarrhoea in weak new born calves, and
- (c) ophthalmia in growing animals.

The existence of these troubles among the cattle of this country is well known and it is probable that malnutrition due to avitaminosis-A is widely prevalent in India. For instance, the incidence of abortion in various dairy herds such as in Quetta, Wellington, etc., and the occurrence of the widespread trouble of "blindness in calves" in Sind and Baluchistan have been shown to be due to vitamin-A deficiency in the ration and these troubles have been reproduced under controlled conditions. A supplement of green feed in the ration prevented the occurrence of both the above troubles in Quetta.

It has been shown previously that the maintenance requirement of carotene for average cattle may be considered to be about 14 mg. per day. A recent analysis has shown that rice straw from three different parts of India did not contain any carotene and the same was also true of various concentrate rations used in our dairy farms. Consequently with a diet based on these concentrates and rice straw as the sole roughage, there is a gross deficiency of vitamin-A in the diet. Various samples of hay have also shown negligible amounts of carotene contents. The importance of choosing proper diets including a good variety of green fodder is, therefore, quite evident and needs no further comment. It is to be remembered here that every green fodder is not an equally rich source of carotene, *jowar*, maize and some varieties of pasture grass being much poorer than clover, lucerne or berseem.

In conclusion, it may be emphasised that the problem of avitaminosis-A in cattle and especially in milch cows has far greater significance than what appears on the surface. It has been pointed out already that the vitamin-A content of milk is dependent on the quality of the feed given to the cow and as milk is probably the most important source of vitamin-A for growing children, avitaminosis-A in cattle is intimately connected with the Public Health problem in India. This aspect of the question was dealt with in detail by Colonel Sir Arthur Olver in his Presidential address to the Medical and Veterinary Research Section of the Indian Science Congress in 1937 under the title "The relation of animal nutrition to Public Health in India" and no further elaboration is, therefore, required at present.

#### SUMMARY

This paper attempts to summarise the present position of avitaminosis-A so far as it relates to the practical feeding of farm animals.

Cereals, grain feeds, vegetable oils and oil-cakes are found to be poor sources of carotene (the precursor of vitamin-A) which is generally associated with the pigments of all plant material.

Green young forage plants are, however, found to be satisfactory sources of carotene for animals.

The vitamin-A requirements of rats, dogs, poultry, swine and cattle are discussed. The excretion, storage and depletion of the vitamin-A reserve of animals through reproduction and milk are described.

The point is stressed that the provision of suitable green feeds and pasture lands is the only practical method of supplying the carotene requirement of farm animals. In this connection, the importance of preserving the vitamin-A potency of green plants when converted into hay or silage has also been emphasised.

The existence of avitaminosis-A in a mild form under the ordinary conditions of stock feeding in India and the need of rectifying this condition are also considered.

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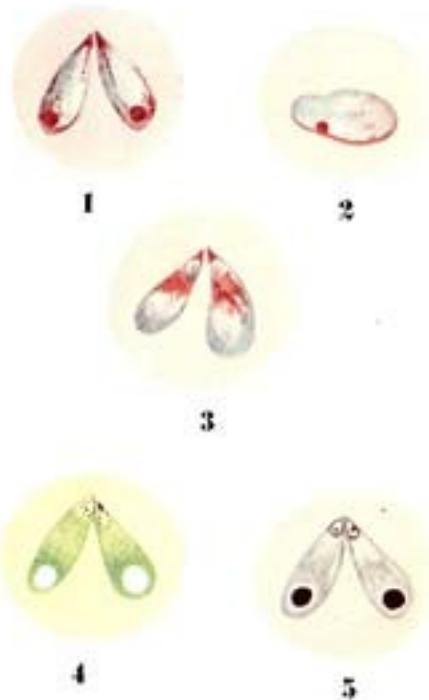
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H. Ray del.

*Babesia bigemina* (Smith and Kilbourne).

All figures are drawn with the aid of *Camera lucida* and the magnification is Ca.  $\times 2,000$ .

Figs. 1-3.—Represent variety of pictures which the red or pink staining material presents when stained with Giemsa (one of the Romanowsky stains).

FIG. 4.—Shows the effect of Fuelgen's reaction on the nucleus counterstained with light green. Note the structure of the nucleus at the apex and the hydrolised vacuolated area at the broad pole of the parasite.

FIG. 5.—Shows a Fuelgen preparation counterstained with Heidenhain's iron-alum-haematoxylin. The hydrolised area is shown to have taken up the haematoxylin stain.

# ON THE NUCLEAR STRUCTURE OF *BABESIA BIGEMINA* (SMITH AND KILBOURNE)

BY

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(Received for publication on the 10th June 1938)

(With Plate IV)

THE family Piroplasmidae to which belongs the genus *Babesia* is placed amongst sporozoa under the order Haemosporidia. But since the publication of Breinl and Hindle's [1908] work on *Babesia canis*, the consideration of a probable relationship of the genus *Babesia* with the flagellate, has become a matter of considerable importance from the systematic point of view. Finding of flagellate forms at some stage of its development in the body of the vertebrate host has, in a way, paved the way for putting forth the arguments in favour of flagellate origin of this genus. Thomson and Fantham [1913] who have successfully cultivated *Babesia canis* *in vitro* did not, however, come across any flagellate stage as shown by Breinl and Hindle.

Dennis [1930] while working on the morphology of *Babesia bigemina*, the causative organism of Texas fever, came to a somewhat similar inference although actual flagellate forms were not encountered by him. An extra-nuclear granule, or blepharoplast and its connection with the nucleus similar to that described for *Babesia canis* has also been shown to be present in *Babesia bigemina* except that in the former a flagellum was found to originate from the granule while in the latter this organelle was absent. Presence of this extra-nuclear granule or 'blepharoplast' was considered by Dennis a feature of sufficient importance to suggest its flagellate affinity.

Smith and Kilbourne [1893] who first described the organism of Texas fever did not give any account of the nuclear structure. Laveran and Nicolle [1890] pointed out that the nucleus was situated at the broad pole of the parasite and that it consisted of a 'karyosome' with a clear area around it. Fantham [1907] gives a detailed description of the distribution of the chromatin\* in this organism. His observations, however, were based on dried smears stained with one of the Romanowsky stains, a method which cannot be relied upon for studying the cytological details. Nuttall and Graham-Smith [1908] describe the nucleus as a small granule with a row of granules extending

\* I put this word in *italics* because any structure that stained pink or red with Romanowsky's stain is usually described as chromatin without paying much attention to its true chemical composition which according to Feulgen (1927) is mainly built upon the thymonucleic acids as its base.

from it; these authors have also shown that the nucleus is situated at the pointed end of the pyriform body. This is of course quite opposed to what has been described by Laveran and Nicolle.

In order to determine what actually is the position of the Indian strain of *Babesia bigemina* with regard to its nuclear structure, detailed observations were made on wet fixed smears. The results of studies incorporated in this article will at once reveal that the structure of the nucleus is quite different from what has hitherto been described. The nucleus is confined to the pointed end of the parasite and consists of 'karyosome', a nuclear membrane, and finely granular chromatin. No indication of any extra nuclear granule or blepharoplast was present in the preparations used for this study.

#### MATERIAL AND METHODS

Material for study was obtained from hill-bulls which showed heavy infection with *B. bigemina* as a result of resuscitation due either to experimental theileriasis or rinderpest. Wet blood smears were fixed in Bouin-Duboscq-Brasil's fluid, alcoholic Bouin's fluid, Schaudinn's fluid and Carnoy's fluid. Smears were fixed for fifteen to twenty minutes except in Carnoy's in which they were kept for five minutes. Stains used were Heidenhains iron-alum haematoxylin, Giemsa and Mallory's triple stain. In order to determine the distribution of chromatin in the nucleus, the method of Feulgen's nucleal reaction was employed. It may be mentioned here that this reaction is based on the hydrolysis of other constituents such as guanine and adenine combined with thymonucleic acid by means of dilute hydrochloric acid at 60°C. and later on obtaining a permanent colour reaction by treating it with basic fuchsine. By this method, so far as is known at present, the proper seat of chromatin in a nucleus can be ascertained with accuracy. Smears treated in this way were counterstained with light green in ninety per cent alcohol. Some of the smears instead of being counterstained with light green were stained in iron-alum-haematoxylin. This method of staining revealed a granule which was invisible in Feulgen preparations. Various pictures of the parasites thus obtained were compared and careful *Camera lucida* drawings were made in each case.

#### OBSERVATIONS

The routine method of staining with Giemsa or Lieshman stain showed so-called chromatin at various places of the pear-shaped organisms and needs little mention from the point of view that dry smears are useless for studying the cytological details. In such preparations a deeply stained pink or red granule appears at the broad pole of the parasite which was interpreted as being a nucleus by Laveran and Nicolle [1908]. Besides this granule, however, a deeply stained area is also seen at the pointed end of the parasite. This was studied in great detail by Nuttall and Graham-Smith [1908] and it was pointed out by them that the nucleus was, truly speaking, situated at the apical end of the organism and consisted of a granule from which extend a number of small granules after the manner of comet (Plate IV, fig. 1). As a matter of fact

this is exactly the picture of the nucleus which one gets so often in dry preparations stained with one of the Romanowsky stains. But it must be said that this holds good only in cases where we are dealing with the typical pear-shaped organism. In rounded or amoeboid forms a single deeply stained granule is usually more prominent than others (Plate IV, fig. 2). In preparations fixed in any of the fixatives mentioned above and stained in iron-alum-haematoxylin shows only a dark dot at the apex of the parasite and sometimes a dark thread-like structure can be seen extending from it. The granule at the broad end which is two to four times as big as one at the apex, also takes up a deep black stain, but in no case was this structure seen to be connected with that thread. Dennis [1930] has also called this bigger granule the nucleus and has shown that it is connected with the smaller one which he refers to as the blepharoplast by means of a rhizoplast. Round this bigger granule he has demonstrated the presence of a nuclear membrane—a structure which unfortunately could neither be seen by Rees [1934] or by the author. It will, however, be seen later on that the bigger granule is devoid of true chromatin and has, therefore, no connection with the nuclear apparatus of the parasite.

In Feulgen preparations the substance contained in the bigger granule at the broad pole is completely hydrolysed and appear as a vacuole when counterstained with light green, while that at the apex gives a definite chromatin reaction (Plate IV, fig. 4). The nucleus in such preparations appears to consist of a deeply stained karyosome usually situated at the extreme narrow end and surrounded by a circle of fine granules. These fine granules may sometimes take up a linear position keeping the karyosome either at its apical, central or lower end. The number of these fine granules varies and are arranged at the periphery of an achromatinic nuclear membrane.

After Feulgen's reaction some smears were stained with iron-alum-haematoxylin. In these preparations the nucleus presented the same picture as mentioned above except that the nuclear membrane was more deeply stained. A very striking feature which was noticed in this preparation was that the area completely hydrolysed by Feulgen reaction and appeared as a vacuole, had taken up a deep black stain (Plate IV, fig. 5). Smears fixed in Carnoy's fluid and stained either with iodine or Best's carmine, however, revealed the true nature of this granule. With iodine it gave a pale brown reaction, while with Best's carmine it appeared as a faintly pink area; suggesting thereby that this area contained certain substance which is allied to glycogen.

It is now clear, therefore, that the deeply staining granule at the broader pole of the pyriform body has no relation with its nuclear apparatus, but instead, is a product of metabolic activity of the parasite. This finding also explains why this granule does not constantly occur in every individual parasite at every stage.

The nucleus in *Babesia bigemina* as described above, is situated at the narrow apical end of the organism and consists of a karyosome surrounded by a circlet of fine granules, arranged at the periphery of the nuclear membrane. With this data at our disposal we can no longer entertain the theory of flagellate origin of this sporozoan as propounded by Dennis [1930].

#### SUMMARY

The nuclear structure of the Indian strain of *Babesia bigemina* has been studied in detail. The nucleus is found to consist of a karyosome surrounded by a circlet of fine granules which give a positive Feulgen reaction. These fine granules are arranged at the periphery of an achromatinic nuclear membrane. The nucleus is situated at the narrow apical end of the pyriform body. A granule which is sometimes seen at the broad pole of the parasite, is two to four times the size of the karyosome and gives a negative Feulgen reaction. With iodine and Best's carmine this granule gives a faintly positive reaction for glycogen.

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FIG. 1.

Section of growth from nose of bullock showing ascospores of rhinosporidium. The ascospores are in close proximity of epithelium.  $\times 60$



FIG. 2.

Section of rhinosporidial growth from the nose of a pony.  $\times 60$

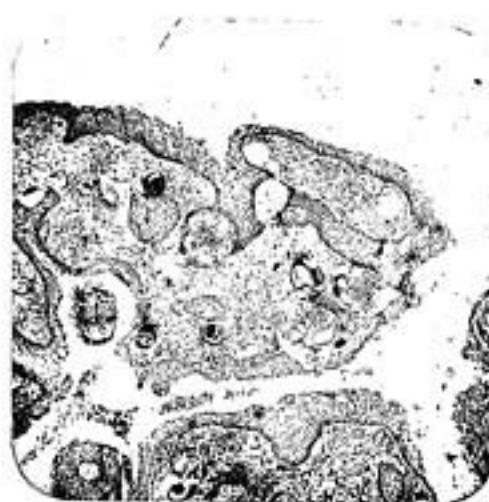


FIG. 3.

Section of rhinosporidial growth from nose of a man.  $\times 60$



FIG. 4.

Section of rhinosporidial growth, Bovine. Note the ascospores bursting through the epithelium.  $\times 60$

RHINOSPORIDIOSIS IN BOVINES IN THE MADRAS  
PRESIDENCY, WITH A DISCUSSION ON THE  
PROBABLE MODES OF INFECTION\*.

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(With Plates V-VIII)

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1. INTRODUCTION

THERE is a remarkable paucity of record of the occurrence of rhinosporidiosis in animals. Only four reports are available from different parts of the world, recording the occurrence of the disease in eight animals. Zschokke [1913] found *Rhinosporidium* in the nasal growth from a horse in South Africa sent to him by Theiler. Fourteen years later Quinlan and de Kock [1926] found the parasite in the nasal growths of two mules in South Africa. Cordero and Vogeslang [1928] reported their finding of the fungus in the nasal

\*Paper read at the Jubilee Session of the Indian Science Congress, held in Calcutta, January, 1938.

growth from a horse in Uruguay. Ayyar [1929 and 1932] in South India detected the fungus in the nasal growths from two bullocks, one cow and one pony. It is, therefore, interesting to note that while this disease has been met with in a small number of equines in different parts of the world, its existence in bovines has been reported, so far, only from the Madras Presidency.

It has been possible for the present author, during the last six years, to record rhinosporidiosis in eighteen bovines and one pony in this presidency, in addition to those already reported by Ayyar [1929]. Ayyar's cases were all from Tanjore district, but the following table shows that the disease exists in other districts as well. The fact that twenty-three cases of rhinosporidiosis (including two equines) in the Madras Presidency have been detected within a period of eight years indicates that the disease is not so rare among animals in this part of India as it is supposed to be. The present writer has not found the disease in cows so far, and most of the cases noted in the table below were detected while examining nasal washings for nasal schistosomiasis.

\*TABLE I

| Serial No. | Year    | Name of District | Name of Town | Kind of Animal | Age            | Seat of Tumour            |
|------------|---------|------------------|--------------|----------------|----------------|---------------------------|
| 1          | 1930    | Chingleput       | Madras       | Bullock        | ..             | Nostrils.                 |
| 2          | 1931    | Malabar          | Calicut      | Bullock        | ..             | Do.                       |
| 3          | 1931    | Ramnad           | Devakottah   | Bullock        | 7 years        | Do.                       |
| 4          | 1932-33 | Tanjore          | Tanjore      | Bullock        | 6 years        | Entrance to left nostril. |
| 5          | 1933-34 | Tanjore          | Tanjore      | Bullock        | Not available. | Nostril.                  |
| 6          | 1934-35 | Tanjore          | Tanjore      | Bullock        | 6 years        | Nostril.                  |
| 7          | 1934-35 | Tanjore          | Tanjore      | Bullock        | 7 years        | Nostril.                  |
| 8          | 1934-35 | Ramnad           | Devakottah   | Bullock        | 5 years        | Nostril.                  |
| 9          | 1934-35 | S. Arcot         | Kallakuruchi | Bullock        | 7 years        | Nostril.                  |
| 10         | 1935-36 | Ramnad           | Devakottah   | Bullock        | 8 years        | Nostril.                  |
| 11         | 1935-36 | Tanjore          | Tanjore      | Bullock        | 7 years        | Nostril.                  |
| 12         | 1935-36 | Ramnad           | Rajapalayam. | Bullock        | 9 years        | Nostril.                  |
| 13         | 1935-36 | Malabar          | Calicut      | Bullock        | 7 years        | Nostril.                  |
| 14         | 1936-37 | Malabar          | Calicut      | Bullock        | 6 years        | Nostril.                  |

\*In this list, the four cases reported by Ayyar (1929 and 1932) have not been included.

| Serial No. | Year    | Name of District | Name of Town | Kind of Animal | Age      | Seat Tumour |
|------------|---------|------------------|--------------|----------------|----------|-------------|
| 15         | 1936-37 | Chingleput.      | Tindivanam   | Bullock        | 8 years  | Nostril.    |
| 16         | 1936-37 | Chingleput.      | Trivellore   | Bullock        | 8 years  | Nostril.    |
| 17         | 1936-37 | Chingleput.      | Saidapet     | Bull-calf      | 2½ years | Nostril.    |
| 18         | 1937-38 | Malabar          | Manjeri      | Bullock        | 4 years  | Nostril.    |
| 19         | 1937-38 | E. Godavari      | Cocanada     | Pony           | aged.    | Nostril.    |

In the Madras Presidency rhinosporidiosis in man has been reported fairly frequently from certain districts and it is interesting to note that the bovines noted above are also from those very districts. So far, all the reported cases of rhinosporidiosis in animals have been from districts other than the Northern Circars; opportunity is now taken to record the first case in a pony at Cocanada, East Godavari.

No cases of rhinosporidiosis in bovines or equines have been reported from Northern India. Allen and Dave [1936] state that at their instance, the Director of Veterinary Services, Central Provinces, issued instructions to his staff to look for rhinosporidial growths in animals in that province but no cases were reported. Those authors made enquiries in the Drug district of Central Provinces and failed to obtain even one infected animal, though they saw over sixty human beings affected with the disease in Raipur and Drug districts. This seems interesting as far as it goes, but compared to what obtains in the Madras Presidency, it seems reasonable to assume that cases of rhinosporidiosis in bovines or equines have escaped diagnosis till now and it may not be long before such cases come to light.

## 2. SITE OF INFECTION

The predilection seat for rhinosporidial growths in animals appears to be the nasal mucous membrane. In all the bovine and equine cases reported so far, such growths were found only in the lower third of the nasal cavity. An analysis of the clinical histories of the affected bovines shows that the growth is either found on the margin of the hole on the nasal septum made for the nose string or by the side of the sessile lesions of nasal schistosomiasis, in the lower third of the nasal cavity. Hence, it would appear that trauma is a necessary factor for the development of rhinosporidial tumours.

The author learns that in certain parts of Northern India, the system of puncturing the nasal septum is not in vogue, and that nasal schistosomiasis exists there. Therefore, there is at least one factor present in causing trauma in the nose in cattle and adequate investigation may result in demonstrating the existence of the disease in bovines in that part of India.

### 3. SEX AND AGE INCIDENCE

As far as the twenty-one bovines included in the present studies are concerned, it is interesting to observe that twenty animals or roughly 95 per cent of the cases were bullocks. In the human beings the incidence of the disease is very much higher in males than in females and it is found to be the case in bovines also. This common sex incidence in bovines and in human beings is somewhat significant and will be discussed later.

The affected animals were all between the ages of four and nine with one exception, a bull-calf (vide Table I), which was reported to be only 2½ years old. Bulls and bullocks are put to the plough when they are about 3½ years of age and their noses are punctured for nose strings when they are about that age. Cows are very rarely used for ploughing in Southern India and their nasal septum is seldom punctured for a nose string.

### 4. DURATION OF THE DISEASE AND RECURRENCE OF THE GROWTHS AFTER EXCISION

It has not been possible to obtain exact data regarding the total duration of the disease in bovines, except in one case, a bullock aged about nine years which was reported to have had the condition for over a period of about five years.

As regards recurrence of the growths after removal, it has been observed from some of the available history sheets that the growths re-appear in about twelve to sixteen months. In a bullock which was kept under observation by the author, the growth did not recur till eleven months after removal. That animal was destroyed and its nasal mucous membrane was sectioned and examined but no *Rhinosporidium* was detected. It would appear, therefore, that recurrence depends on whether or not, the diseased tissue has been removed completely along with the adjoining healthy tissue.

### 5. CONCURRENT INFECTION

Out of the fifteen animals whose histories are known eight (or 54·4 per cent) had nasal schistosomiasis as well.

### 6. CLINICAL FEATURES

The tumours caused by *Rhinosporidium* in bovines and equines can be classed under two types, viz. (1) Pedunculated and (2) Sessile. The size of the growths is seldom bigger than a large playing marble. The sessile types of tumours are more or less flat and are usually associated with the lesions of nasal schistosomiasis and appear to be seldom bigger than a double bean. The growths appear more or less lobulated, soft and spongy. The surfaces of both types of tumours are dotted over with small white specks which represent the sporangia or asci of *Rhinosporidium*. The growths are pink or reddish in colour and bleed readily when handled. It is interesting to note that in all the cases observed so far, the lesions were unilateral in the nose.



FIG. 1.

A small growing ascus. Note the nucleus as a ring with the nucleolus which is eccentric.  $\times 700$

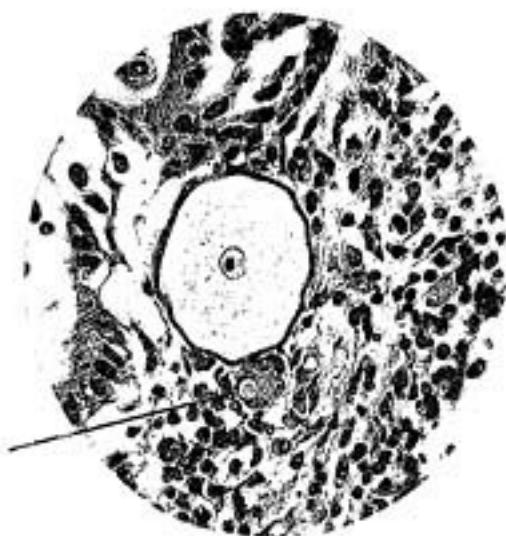


FIG. 2.

Developing ascospores (Trophozoites). Note the small one at the bottom of the bigger one. Note the granular protoplasm, the nucleus and the nucleolus.  $\times 700$

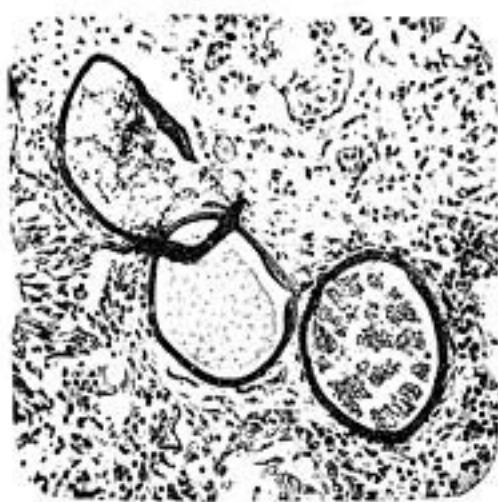


FIG. 3.

The central ascus has a thick wall in which the "pore" is seen. Note the nuclear divisions in the protoplasm. The ascus on the right shows that condensation of protoplasm round nuclear bits is in progress.  $\times 200$

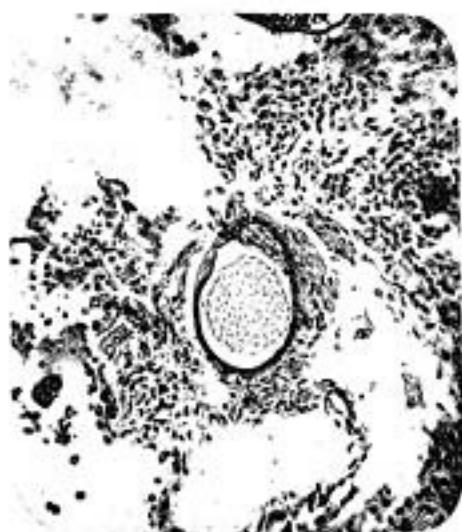


FIG. 4.

Section of rhinosporidial growth - equine showing a developing ascus with a thick wall in which is seen the pore.  $\times 200$



FIG. 1.

Section of growth - bovine. A developing ascus in which the nuclear division is completed.  $\times 200$



FIG. 2.

Section of rhinosporidial growth - bovine. A young ascus in which condensation of protoplasm around bits of nuclei is just commencing. Note the pore on the wall of the ascus.  $\times 200$



FIG. 3.

A degenerating ascus, the contents of which have been replaced with tissue cells. Note the section of a giant cell inside it.  $\times 200$

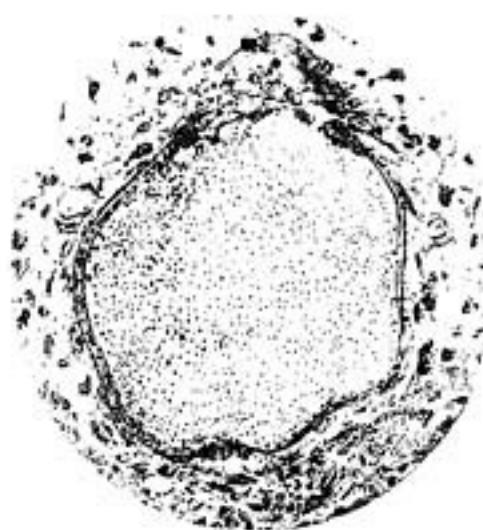


FIG. 4.

An ascus filled with small spores only.  $\times 450$

In bovines suffering from the disease, the symptoms simulate those of nasal schistosomiasis. The presence of nasal discharge and snoring noise during breathing are found in both the conditions, hence clinical diagnosis may not be easy, excepting when the lesions of *Rhinosporidium* alone exist, in which case, the nasal discharge may be unilateral. Diagnosis is easy with the aid of the microscope under which spores or sporangia of *Rhinosporidium* are seen in the nasal discharge. This similarity of the clinical symptoms of the two diseases of the nose perhaps accounts for the non-recognition of the condition in bovines in countries where nasal schistosomiasis exists. All the cases in Southern India were diagnosed microscopically in the laboratory.

#### 7. HISTOPATHOLOGY AND LIFE-HISTORY OF THE ORGANISMS

In a section of the rhinosporidial growth papillary processes of the epithelium are seen. Under the epithelium is found a stroma of delicate fibrous or fibro-myxomatous tissue in which are present the sporangia or asci of *Rhinosporidium*. This sub-epithelial tissue is very vascular with young or newly developed capillaries. In addition, one finds infiltration with polymorpho-nuclear cells, lymphocytes, plasma cells, eosinophiles and red blood cells in the tissue. Giant cells are also met with though rarely.

In the crypts, formed between the papillary processes, are seen morular spores embedded in the nasal mucus. Those spores evidently were discharged by the asci. The asci appear to be more numerous under the epithelium and the fully developed ones are usually just below it. The large asci under the epithelium seem to press upon that tissue which yields to pressure from below and ultimately ruptures, thus enabling the mature ascus to discharge its spores with the nasal mucus outside the animal body. Very few of the mature asci appear to liberate their spores in the soft tissue in which they develop and this fact is evident from their ruptured walls and the number of young sporangia seen, particularly in the vicinity of the mature ascus. Though mature asci contain a large number of spores, yet it is interesting to note that the number of young sporangia is very much less than the number of spores which may have been discharged in the soft tissue of the tumour. The only hypothesis that can be advanced to account for the small number of sporangia, compared with the number of spores liberated, appears to be the presence of antagonistic body cells and their lysins which destroy most of the spores. The smallest developing trophozoite is rounded or ovoid and measures about 6 to 8  $\mu$  and is filled with granular protoplasm in which is present a nucleus with a nucleolus. It is interesting to note that the smallest developing trophozoite resembles the small sized spore in an ascus, a reference to which will be made later. A study of the developing trophozoites in sections reveals exactly what has been described by Ashworth [1923] and the details need not be entered into here. A fully grown ascus is rounded or slightly oval and measures 250 to 300  $\mu$  in diameter. The thickness of the wall varies in mature asci in proportion to the size of the ascus, the mature ones having comparatively thin walls. In some young asci the so-called "pore" through which

the spores are said to escape, is seen in the wall depending upon the orientation of the ascus in section. The spores in an ascus vary in their sizes; some of them are large, and each large spore has a capsule containing a nucleus and proteinaceous granules and the rest are small spores with no capsule and contain finely granular protoplasm and a nucleus. The large spore measures 8 to 10  $\mu$  and the small one 5 to 7  $\mu$ .

### 8. IDENTITY OF THE PARASITE

It is interesting to note that rhinosporidiosis in cattle occurs in such areas in the Madras Presidency where it is known to exist in man. There appears to be no difference in the histopathology of the lesion or in the morphology of the fungus in the material obtained either from man, bovine or equine. Wenyon [1926] doubts the existence of any specific differences between equine and human rhinosporidiosis. Ayyar [1932] pointed out that there is a very close resemblance between the human and bovine lesions as regards the site of growth, gross appearance of the tumour, its histology and the morphology of the sporangia, etc. He sent some of his material to Ashworth who confirmed those findings and said that the discovery of *Rhinosporidium* in bovines may throw light on the mode of infection. Allen and Dave [1936] also said that the sections of rhinosporidial lesions from cattle looked very similar to those from man. The author could not find any specific difference in the morphology of the causal organism or in the histopathology of the lesions in men, bovines or equines. It would appear, therefore, that the causal organism of rhinosporidiosis in man and animals is identical. This conclusion seems to throw light on the mode of transmission as prophesied by Ashworth and will be discussed later.

### 9. DISSEMINATION OF SPORES FROM THE LESIONS

The mature ascus may burst upon the nasal mucous membrane liberating the spores on its surface or it may burst in the soft tissue. In the former case the spores get mixed up with the nasal mucus and may be passed outside the animal body, or as it usually happens in cattle or even in human beings, they may be swallowed with the nasal mucus to find their way outside the body through the faeces. If the ascus bursts inside the tissue, the spores remain in the tissue having to complete their destiny there alone. To complete their biological destiny outside the animal body, the spores are for obvious reasons provided with protection and food material which on the other hand, are not required by those that are destined to complete their life-history in the tissue, where protection and food are provided for by the host tissue. It is interesting to note that the author found in the nasal discharge only the large type of spores each having proteinaceous granules and a capsule. Other workers with material obtained from human patients, particularly Allen and Dave [1936] have recorded the presence of only the large spores in the nasal discharge which is diagnostic of the infestation. Ashworth [1923] and Karunaratne [1936] said that the smallest developing trophozoite in the tissue measures

about six to seven microns and there are no proteinaceous granules present in it which, they think, were utilised for building up the cyst wall. It is not clear why they should think that in the host tissue, where food material is available, the spore should utilise its own proteinaceous granules and shrink in size only to grow bigger again at the expense of the host. It is doubtful if such a thing happens in nature. Again the size of the young trophozoite is almost that of the small spore found in an ascus and this observation, therefore, suggests that the smaller type of spore alone develops into a trophozoite in the host tissue. The fact that the large spore is provided with proteinaceous food granules and is protected with a fairly thick chitinous capsule at once suggests that it is destined to complete its life-history outside the animal body, even as a seed is provided with reserve food material and a testa.

Beatty [1906] considered that the "spore morulae" which are protected by their envelopes may undergo changes outside the body. Some workers are of opinion that the small spores in an ascus are degenerate forms, but a careful examination of such spores shows that each has a nucleus and protoplasm, the staining reactions of which do not suggest degeneration. Hence it would appear that they really are living spores destined to continue the parasitic development in the tissue of the host, and the large spores are destined to complete their biological destiny outside the animal body.

#### 10. ATTEMPTS TO CULTURE *RHINOSPORIDIUM*

Ashworth [1923] concluded that *Rhinosporidium* is a fungus and placed it among the *Phycomycetes* and this has been accepted by Ramsbottam [1931]. Dodge [1936] quotes Wernicke [1907] and places it among the *Coccidioidaceae*. Excepting for a partial success of Rettle, quoted by Ashworth, no one seems to have succeeded in cultivating the organism on artificial media in the laboratory. The fact of the difficulty experienced in cultivating that fungus indicates the delicate nature of the organism. This feature is further supported by the fact that cases of *Rhinosporidium* are few and far between being localised only to certain areas as far as could be gathered from the published records. It is apparent that the very first case of rhinosporidiosis could have occurred only from a pre-existing fungus outside the animal body. Further, indigenous cases of rhinosporidiosis have been reported from countries very widely separated from one another, viz., India, Union of South Africa, Philippine Is. and South America, etc., and for obvious reasons such cases could have received the infection only from a pre-existing saprophytic form of the fungus in those localities. Again it is interesting to note that in India rhinosporidiosis is usually found in the agricultural class of people and in cattle. This fact suggested to the author that in nature a saprophytic form of the fungus probably germinates and thrives in bovine dung since bovines swallow their nasal discharges and with them the spores of *Rhinosporidium* when the lesions are in the nose. The possibility of the large spores being destined for a phase of life outside the body of the animal has been discussed above. With this idea in view, a diseased animal was obtained by the author from Tanjore for experimental purposes. This animal had a very small growth, about the size of a

pea, on the margin of the hole in the septum of the left nostril. Pellets of faeces from that animal were distributed in sterile petri-dishes and kept at room temperature, and also at 37°C., for varying periods to study the fungi developing in them. The study was continued, for a period of over four months and the number of different kinds of fungi obtained during the period was so great as to render it overwhelmingly difficult to cope with the work of identifying them. This method was given up in preference to the direct method of planting, on sterile dung, small pieces of growth or the ascii obtained by teasing pieces of growth. This method proved to be encouraging since it was found possible to obtain the same kind of fungus in a series of experimental cultures while the best growth was obtained invariably at the room temperature. These cultural experiments were extended to material obtained from human beings and again a fungus resembling the one in cultures of bovine material, was obtained every time. A number of ascii, obtained from teased pieces of growths, were treated with five per cent formalin for three minutes, washed in several changes of sterile normal saline and sown on sterile cow dung. These germinated quite satisfactorily though slowly. Once the fungus appeared on the dung, there was little difficulty in obtaining sub-cultures on Sabaraud's medium with dung juice in it. The best medium for sub-culture was found to be horse-dung-agar with gland juice smeared over it or added to it. The growth on the latter appeared to be more vigorous than on any other media.

On sterile dung it took nearly two weeks to show a white globular fluffy growth. It was interesting to watch the growth in a single ascus planted on dung. In about a week after planting, the wall of the ascus turned dark in colour and a few small, white, ascicular projections appeared round about the ascus at its point of contact with the dung. In about a fortnight the whole ascus looked like a fluffy ball of white wool about the size of a mustard seed. At the end of four weeks it was about the size of a millet seed but flattened showing a few beads of moisture on the surface. In about six weeks a number of small grape-like rounded white bodies appeared on the surface and microscopic examination showed that these were composed of a thick felted mass of mycelia and no fructification had occurred. The fungus has been sent to experts for identification and the report is awaited. The fungus obtained from the culture has been implanted into the punctured hole of the nasal septum in a bull-calf and the result is awaited. The description of the fungus and the result of animal inoculation will, it is hoped, form the subject of another paper.

#### 11. PROBABLE MODES OF INFECTION

Ashworth [1923] refers to direct transmission of the discharged spores from the lesions of *Rhinosporidium* as an obvious method of spread from man to man, but such direct transmission of the disease has failed in the hands of workers who used laboratory animals for the purpose. There is evidence to show that personal contact even for a number of years does not result in transmission. A good example of failure of direct transmission, by such personal contact, is the one reported by Kurup [1931] of a man, with lesions in his

pharynx, living with his wife and family for a period of over eight years without infecting any one of them. The present author has repeatedly failed to transmit the disease directly, with the spores from the lesions of diseased bovines, to healthy bovines or laboratory animals. Hence it would appear that in nature direct infection with large spores from the lesion does not take place. If the discharged spores obtained from the parasitic stage of *Rhinosporidium* are not infective, the only other alternative is to admit the existence of a saprophytic form of the fungus whose spores are infective.

It would be interesting to examine this question of the existence of *Rhinosporidium* outside the animal body as a saprophyte. An analysis of the published reports on the disease by workers in India,—notably Wright [1922], Kurup [1931], Noronha [1933], Allen and Dave [1936] and Mandlik [1937]—shows that there is a close relationship between rhinosporidiosis and agriculture. A very large number of cases reported, belong to the agricultural class of people in India. Allen and Dave say that the condition exists in the agriculturists even outside India. The present author found that over 95 per cent of the bovines affected were bullocks used for agricultural operations, chiefly ploughing. This finding of the author supports the view that rhinosporidiosis is primarily an occupational disease. Secondly it seems clear that the infection is carried either by (a) dust or by (b) water.

(a) *Infection dust-borne*.—In the recorded cases of rhinosporidiosis in human beings over 90 per cent had lesions in the nose. In the series recorded by Allen and Dave, 68·3 per cent were agriculturists and their children, of which over 97 per cent had the lesions in the nose. Among bovines or equines recorded, 100 per cent had the lesions in the nose. The enormous percentage of infection in the nose in men and animals connected with agriculture at once suggests that the infective material is dust-borne and that it should have existed on the fields.

When ploughing fields, plenty of dust is raised and the man behind the plough gets more of it than the animals in front which draw the plough. Therefore such men will be exposed to the infective material much more than the bullocks and this is supported by the records which show that the incidence in man is very much higher than in bullocks. Women and cows, seldom if at all, take part in ploughing lands and the incidence in them is negligible. Hence it seems reasonable to suggest that the common causal factor of infection is on the fields and is raised with the dust while ploughing and is inhaled. Perhaps, therefore, the infection is a nasal condition than of any other tissue. It is possible that infective material reaches the fields through manure in which a variety of fungi grow. Cattle swallow their nasal discharge, and with it a large number of spores from nasal lesions if present. These spores pass out with their faeces in the same way as the other spores of fungi pass out when they are ingested along with food or fodder. Most of such faeces of the diseased animals are thrown on the manure heap and some of the spores of *Rhinosporidium* in them are likely to germinate into saprophytic fungus, fructify and liberate spores. If such manure is thrown on the fields, there is every chance of those spores getting inhaled with the dust while ploughing. Such spores

get arrested in the mucus present in the nose of man or animals, and under favourable circumstances attack the nasal mucous membrane and produce lesions.

Man has also got the habit of swallowing a part of his nasal discharge particularly when it reaches the posterior nares and if one has rhinosporidial lesion in his nose, he will swallow spores along with the nasal discharge. Such spores naturally pass out through faeces. In villages, people defaecate on the fields or other defaecating areas near tanks, nullahs, etc. Further, some people have the habit of blowing their noses so as to throw the nasal discharge on the ground and with it spores will also be thrown out if that person has nasal lesions. Some of those spores, whether in the faeces or in the nasal discharge, may germinate into the saprophytic infective forms, given the time, optimum temperature and moisture. Those may be primary or additional sources from which the infective material is disseminated through dust or through stagnant water to which surface washings from infected areas gain access during the rains. (It is probable that soiled fingers also carry the infection to the nose, eye, etc., in the human being).

(b) *Is the Infective material water-borne?* Mandlik [1937] observes that rhinosporidial infection in Poona and its neighbourhood is localised to areas divisible into groups and that the infection in these groups is localised to infected water of certain wells, tanks or infected sections in the river course. He holds that the spores protected with the stout chitinoid envelopes, escape with the nasal discharge into the water of tanks or pools when bathers with rhinosporidial lesions swim in them and those spores infect later some of the unlucky persons that swim or dive in such water. In other words, according to Mandlik, the infection is direct, and direct transmission with such spores has been negatived by experimental evidence. Hence the water should have become infective in some other way. It has been pointed out above that surface washings, and along with them spores of the saprophytic form of the fungus may gain entrance to more or less stagnant water of tanks, wells, etc., from arable lands or defaecation areas. Mandlik [1937] says that the tanks, etc., which he found infective are subject to inundation during rains. Hence it is reasonable to suppose that tanks, etc., may receive infective material from surface washings.

Thus, it would appear that infection through dust and water can well be explained if the existence of a saprophytic form of the fungus is admitted, the supposition of which is not unreasonable, since direct infection with spores from the lesion is not possible.

#### 12. SUMMARY

1. Nineteen cases of rhinosporidiosis in the nose of animals, consisting of eighteen bullocks and one pony are now recorded in addition to four animals recorded by Ayyar.

2. Rhinosporidiosis in a pony in the Northern Circars is recorded for the first time.

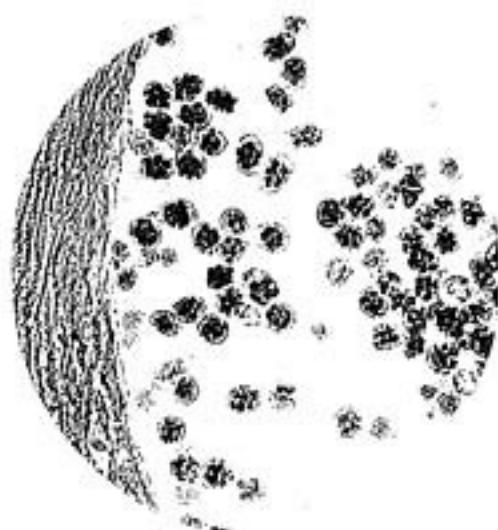


FIG. 1.

The large spores in asci of rhinosporidium. Note the protuberant granules and the capsule of each spore.  $\times 750$



FIG. 2.

Large spores in nasal discharge. Note the thicker capsule than in those in asci of the lesion.  $\times 650$



3. It is observed that the disease is prevalent both in man and animals in certain districts of the Madras Presidency.

4. The organism causing the lesions in man and bovines is probably identical.

5. In cows, as in women, the disease is rarely found, but in men and bullocks that take part in ploughing, the incidence of the disease is high.

6. The probability of the existence of a saprophytic form of the fungus is suggested and discussed.

7. The possibility of dust and water being the medium through which infection may be carried to healthy individuals is discussed.

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# STUDIES ON A NATURAL OUTBREAK OF PIGEON-POX

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(With Plates IX and X)

## INTRODUCTION

THERE are few authentic records of the occurrence of bird pox in India. Since 1934 suspected cases have been examined at this Institute and it is now possible to state that the disease is a menace to the poultry industry. Apparently pigeon-pox has not been described in India, and this paper records the only outbreak that has come to our notice.

In April, 1936, spontaneous cases of what later proved to be pigeon-pox occurred among the healthy stock of pigeons at this Institute. We had then fifty-eight pigeons of which twenty-eight were isolated as sick and kept under close observation for study. Six pigeons died and the rest recovered.

Previous to the occurrence of this outbreak, studies on pigeon-pox and fowl-pox with reference to their propagation *in vivo*, the preparation of a vaccine from the pigeon-pox virus for use against fowl-pox and routine diagnostic work on the specimens suspected for fowl-pox were in progress and are being continued. The seed materials for our stock strains of fowl-pox and pigeon-pox viruses were obtained in 1934 through the courtesy of the Veterinary Laboratory, Ministry of Agriculture, Weybridge, England, and an Indian strain of fowl-pox was later added in 1936.

Marx and Sticker [1902] found that the causative agent of pigeon-pox in a natural case was a filterable virus which could be successfully passaged through pigeons but when passaged through the fowl, it became attenuated for the pigeon even after the first passage. M'Fadyean [1908] reported that in outbreaks of 'epithelioma contagiosum' various species of domesticated poultry and pigeons were affected. Dean and Marshall [1909] confirmed the filtrability of the causative virus in a natural outbreak of 'pigeon diphtheria'. Jowett [1909] considered that the bird-pox virus would adapt itself to the particular species of birds in which it might have become implanted, losing its power to infect any of the other species. Haring and Kofoid [1911-12] reported that fowls could be infected with pigeon-pox and the sera of such infected fowls could fix the complement to a greater extent than that of normal

fowls, using extracts of liver or the lesion of the infected fowl as the antigen. Hutyra and Marek [1926] reported that continued passages of pigeon-pox through fowls attenuated the virus to an extent of avirulence. Ward and Gallagher [1926] mentioned that pox could be readily transmitted from pigeon to the fowl but great difficulty was experienced in attempting the reverse transmission. Doyle and Minett [1927] showed that the virus of fowl-pox and pigeon-pox were immunologically indistinguishable but they differed somewhat in their species adaptation. However, they eventually succeeded in adapting the fowl-pox virus to pigeon's skin through successive passages.

Doyle [1930] made a notable addition to the field of immunology by showing that pigeon-pox virus protected fowls against subsequent infection with fowl-pox virus but he was unable to demonstrate any neutralizing antibodies in the sera of the immunized birds. He also observed that the virulence of pigeon-pox virus for the fowl could be increased by passage through fowls finally acquiring all the characters of fowl-pox virus. Various other workers, Findlay [1930], Brandy and Bushnell [1932], Canham [1932], Burnet [1933] and Irons [1934] have also worked on this disease and their results have been almost similar to those of Doyle and Minett [1927] and Doyle [1930]. The results obtained by Michael [1932] and Johnson [1935] with the use of glycerinated pigeon-pox virus as a vaccine against fowl-pox, however, were not quite in agreement with those of Doyle [1930].

#### CLINICAL DESCRIPTION OF THE NATURALLY AFFECTED PIGEONS

The affected pigeons were dull with ruffled feathers. Their appetite in some cases was impaired. Fever was present in some of the birds prior to the onset of lesions. Wart-like small pea-sized nodular growths attached<sup>2</sup> to the skin over the eyelids, cheesy diphtheritic lesions in the buccal cavity (Plate IX, Figs. 1 and 2), varying degree of conjunctivitis and marked reduction of the body weight characterised the disease. Signs of gastro-enteritis with greenish liquid faeces soiling the feathers beneath the cloaca were seen on autopsy. Mortality was 21·4 per cent. In most of the cases lesions disappeared and birds recovered after about three or four weeks. The period of incubation in spontaneous cases could not be determined but in artificially infected birds it varied from two to five days.

#### METHOD OF EXPERIMENTAL TRANSMISSION

The material used for experimental transmission of the disease consisted of the cutaneous lesions from the eyelids of the affected pigeons, gently removed with a pair of forceps and desiccated *in vacuo* over phosphoric anhydride using a negative pressure of about 30 cm. of mercury. The period of drying lasted for about eighteen hours. The crusts when dried were pulverised with a pestle and mortar and allowed to soak in a little normal saline solution for an hour. More saline was added to make a homogenous emulsion with a final concentration of one per cent. It was inoculated over the feather follicles of a leg after pulling out a few feathers, by gentle application with a camel hair brush or a cotton wool swab.



FIG. 1.

A natural case of pigeon pox showing buccal diphtheritic lesions, wart-like nodular growths on the upper eyelid and signs of diarrhoea.



FIG. 2.

Head and neck of the same pigeon as in Fig. 1 showing the buccal and cutaneous lesions (magnified)



Material from the buccal lesions has not been used for subinoculation purpose because it has been established in another paper by the authors [1936] that in an identical condition in Indian fowls the causal agent of the buccal diphtheritic lesions and the cutaneous lesions is one and the same filterable virus.

#### TRANSMISSION EXPERIMENTS

(a) *Experimental transmission of the disease in pigeons.*—Five apparently healthy pigeons that were in contact with the natural cases of pigeon-pox were soon separated and inoculated on 20th April 1936 with the Indian strain of pigeon-pox virus as a preliminary experiment (Table I). It took some weeks before a fresh lot of healthy pigeons could be obtained. Pox lesions developed only in Pigeon No. 3 and the remaining four pigeons resisted the infection, probably due to the varying degree of immunity which might have developed as a result of their having been in contact with the naturally infected cases before they were segregated or due to their being naturally immune to this infection as considered by Findlay [1930].

Three weeks after the above experiment, Indian strain of this virus after passage through Pigeon No. 3 was subinoculated into five healthy pigeons on 12th May 1936. Of these, four readily took the infection and in one (Pigeon No. 45) there was no reaction (Table I).

Mixed crusts from Pigeons Nos. 41, 42, 43 and 44 were further passaged into twelve healthy pigeons on 13th June 1936 with the result that experimental disease was reproduced in ten pigeons and two were found to be resistant (Table I). Subsequent passages of the Indian strain of this virus were continued in pigeons for the availability of the material for further work.

Lesions in the experimentally infected pigeons were mostly confined to the seat of inoculation. Some of these birds developed acute gastro-enteritis and succumbed and one showed eye lesions as well.

TABLE I  
*Experimental transmission of the disease in pigeons*

| Pigeon No. | Date of inoculation | Inoculum (Indian strain of pigeon-pox from natural cases) | Result      | Remarks   |
|------------|---------------------|---|-------------|---|
| 1          | 20th April 1936     | First passage   | No reaction | Originally in contact with natural cases of pigeon-pox. |
| 2          | 20th April 1936     | Do.   | Do.         | Ditto.  |
| 3          | 20th April 1936     | Do.   | Reacted     | Ditto.  |



TABLE I—*contd.*  
*Experimental transmission of the disease in pigeons—contd.*

| Pigeon No. | Date of inoculation | Inoculum (Indian strain of pigeon-pox from natural cases) | Result        |   | Remarks   |
|------------|---------------------|---|---------------|---|---|
| 4          | 20th April 1936     | First passage .   | No reaction . | . | Originally in contact with natural cases of pigeon-pox. |
| 5          | 20th April 1936     | Do. . .   | Do. . .       | . | Ditto.  |
| 41         | 12th May 1936       | Second passage .  | Reacted .     | . |   |
| 42         | 12th May 1936       | Do. . .   | Do. . .       | . |   |
| 43         | 12th May 1936       | Do. . .   | Do. . .       | . | Died on 1st June 1936.                                  |
| 44         | 12th May 1936       | Do. . .   | Do. . .       | . | Died on 6th June 1936.                                  |
| 45         | 12th May 1936       | Do. . .   | No reaction . | . |   |
| 46         | 13th June 1936      | Third passage .   | Reacted .     | . | Died on 30th June 1936.<br>(showed eye lesions).        |
| 47         | 13th June 1936      | Do. . .   | Do. . .       | . |   |
| 48         | 13th June 1936      | Do. . .   | Do. . .       | . |   |
| 49         | 13th June 1936      | Do. . .   | Do. . .       | . |   |
| 50         | 13th June 1936      | Do. . .   | Do. . .       | . |   |
| 51         | 13th June 1936      | Do. . .   | Do. . .       | . | Died on 27th July 1936.                                 |
| 52         | 13th June 1936      | Do. . .   | Do. . .       | . | Died on 19th July 1936.                                 |
| 53         | 13th June 1936      | Do. . .   | Do. . .       | . | Died on 1st July 1936.                                  |
| 54         | 13th June 1936      | Do. . .   | No reaction . | . |   |
| 55         | 13th June 1936      | Do. . .   | Do. . .       | . |   |
| 56         | 13th June 1936      | Do. . .   | Reacted .     | . |   |
| 57         | 13th June 1936      | Do. . .   | Do. . .       | . | Died on 6th February 1936.                              |

(b) *Experimental transmission of the disease in fowls.*—Three fowls were at first inoculated with natural pigeon-pox material on 20th April 1936,

(Table II). There was only a transient local congestion of the feather follicles. Although the reaction was not very suggestive of successful transmission of the disease from pigeons to the fowls, yet, their subsequent immunity test against fowl-pox virus showed that they had developed the immunity. Doyle and Minett [1927], Findlay [1930] and Irons [1934] obtained similar results by the inoculation of fowls with pigeon-pox virus. Two control fowls Nos. 56 and 57 were inoculated with fowl-pox virus alone. They reacted typically unlike the three fowls that received the Indian strain of pigeon-pox virus previously.

Four more fowls that were inoculated with the Indian strain of pigeon-pox virus from Pigeon No. 3, (Table I) on 13th June 1933, were tested with our stock fowl-pox virus, along with two healthy controls on 25th June 1936 (Table II). All the fowls that received a dose of the Indian strain of pigeon-pox virus reacted mildly to it and later resisted the test dose of fowl-pox virus which produced the disease in the two healthy controls. This showed that the Indian strain of pigeon-pox virus, like the English strain of the same virus, can be used as a live vaccine against fowl-pox.

TABLE II

*Experimental transmission of the disease in fowls and cross immunity tests with fowl-pox virus*

| Fowl No. | Date of inoculation with the Indian strain of pigeon-pox virus | Result of the inoculation | Date of immunity test with fowl-pox virus (English strain) | Result of immunity test | Remarks  |
|----------|--|---------------------------|--|-------------------------|----------|
| 28       | 20th April 1936  | Mild reaction             | 6th May 1936   | No reaction             |          |
| 29       | 20th April 1936  | Do.                       | 6th May 1936   | Do.                     |          |
| 30       | 20th April 1936  | Do.                       | 6th May 1936   | Do.                     |          |
| 56       | ..   | ..                        | 6th May 1936   | Reacted                 | Control. |
| 57       | ..   | ..                        | 6th May 1936   | Do.                     | Do.      |
| 88       | 13th June 1936   | Mild reaction             | 25th June 1936   | No reaction             |          |
| 89       | 13th June 1936   | Do.                       | 25th June 1936   | Do.                     |          |
| 90       | 13th June 1936   | Do.                       | 25th June 1936   | Do.                     |          |
| 91       | 13th June 1936   | Do.                       | 25th June 1936   | Do.                     |          |
| 104      | ..   | ..                        | 25th June 1936   | Reacted                 | Control. |
| 105      | ..   | ..                        | 25th June 1936   | Do.                     | Do.      |

## FILTRATION EXPERIMENTS

One per cent saline emulsion of the cutaneous crusts after autolysis at 37°C. for one hour and centrifugation was filtered through an unused Berkefeld 'V' candle and *Pasteurella avisepticus* was added to the virus emulsion before filtration to control the safety and efficacy of the filter. The filtration was done under negative pressure at 56 cm. of mercury and it took about thirty minutes to deliver about 25 c.c. of the filtrate. The filtrate, as obtained above, was tested for bacterial sterility and was found to be sterile. It was swabbed over the feather follicles of five healthy pigeons (Table III). Five more pigeons were then inoculated with the unfiltered emulsion to serve as controls. Four out of the five pigeons inoculated with the filtrate reacted typically and in one, Pigeon No. 38, there was no reaction. Out of the five control pigeons, inoculated with the unfiltered material, four reacted and one was found to be resistant.

TABLE III  
*Filtration experiments*

| Pigeon No. | Date of inoculation | Inoculum               | Result of the inoculation |
|------------|---------------------|------------------------|---------------------------|
| 36         | 12th May 1936       | Berkefeld 'V' filtrate | Reacted.                  |
| 37         | 12th May 1936       | Do.                    | Do.                       |
| 38         | 12th May 1936       | Do.                    | No reaction.              |
| 39         | 12th May 1936       | Do.                    | Reacted.                  |
| 40         | 12th May 1936       | Do.                    | Do.                       |
| 41         | 12th May 1936       | Unfiltered emulsion    | Do.                       |
| 42         | 12th May 1936       | Do.                    | Do.                       |
| 43         | 12th May 1936       | Do.                    | Do.                       |
| 44         | 12th May 1936       | Do.                    | Do.                       |
| 45         | 12th May 1936       | Do.                    | No reaction.              |

## CROSS IMMUNITY TESTS

(a) *Testing the immunity of pigeons, immunized with the English strain of pigeon-pox virus, against the Indian strain of the same virus.*—Five pigeons immune to the English strain of pigeon-pox virus were tested with the Indian





FIG. 1.

Pigeon-pox lesions reproduced with the Indian strain of pigeon-pox virus on the leg of Pigeon No. 315, previously immunized with the English strain of the same virus on the other leg.



FIG. 2.

Pigeon-pox lesions reproduced with the Indian strain of pigeon-pox virus on the leg of Pigeon No. 318, previously immunized with the English strain of the same virus on the other leg.

strain of the same virus on 20th April 1936 (Table IV). Pigeon No. 344 resisted both the strains and was, therefore, naturally immune to this infection, whereas, the other four pigeons reacted typically to the Indian strain of the virus which suggested that there was some immunological difference between the two strains (Plate X, Figs. 1 and 2).

Four more pigeons, after recovery from experimental infection with the English strain of pigeon-pox virus were submitted to immunity test against the Indian strain of the same virus on 12th May 1936 (Table IV). Of these, three pigeons showed no reaction but the fourth one, No. 35, reacted mildly.

Since the results of the above two experiments were not similar, a fresh lot of four pigeons which were previously infected with the English strain of pigeon-pox virus were tested with the Indian strain of the same virus on 23rd May 1936 (Table IV). Pigeon No. 12 died three days after the cross inoculations due to other causes and the other three proved refractory.

In continuation of this work seventeen more pigeons, actively immune to the English strain of pigeon-pox, were inoculated with the Indian strain of the same virus on 13th July 1936. Of these, fifteen pigeons reacted and the remaining two showed no reaction.

From the results of the above experiments it appears that cross immunity does not always exist between the English and the Indian strains of pigeon-pox virus and there seems to exist some difference in the antigenic complex of the two strains.

TABLE IV

*Cross immunity test on pigeons, immune to the English strain of pigeon-pox virus against the Indian strain of the same virus*

| Pigeon No. | Date of inoculation with the English strain of pigeon-pox virus | Result of the inoculation | Date of cross immunity test with the Indian strain of pigeon-pox virus | Result of the cross immunity test | Remarks |
|------------|---|---------------------------|--|-----------------------------------|---------|
| 344        | 6th March 1936  | No reaction .             | 20th April 1936  | No reaction .                     |         |
| 345        | 6th March 1936  | Reacted .                 | 20th April 1936  | Reacted .                         |         |
| 346        | 6th March 1936  | Do. .                     | 20th April 1936  | Do. .                             |         |
| 348        | 6th March 1936  | Do. .                     | 20th April 1936  | Do. .                             |         |
| 642        | 6th March 1936  | Do. .                     | 20th April 1936  | Do. .                             |         |
| 32         | 20th April 1936   | Do. .                     | 12th May 1936  | No reaction .                     |         |
| 33         | 20th April 1936   | Do. .                     | 12th May 1936  | Do. .                             |         |

TABLE IV—*contd.*

*Cross immunity test on pigeons, immune to the English strain of pigeon-pox virus against the Indian strain of the same virus*

| Pigeon No. | Date of inoculation with the English strain of pigeon-pox virus | Result of the inoculation | Date of cross immunity test with the Indian strain of pigeon-pox virus | Result of the cross immunity test          | Remarks |
|------------|---|---------------------------|--|--|---------|
| 34         | 20th April 1936   | Reacted                   | 12th May 1936  | No reaction                                | .       |
| 35         | 20th April 1936   | Do.                       | 12th May 1936  | Mild reaction                              | .       |
| 9          | 20th April 1936   | Do.                       | 23rd May 1936  | No reaction                                | .       |
| 10         | 20th April 1936   | Do.                       | 23rd May 1936  | Do.  | .       |
| 11         | 20th April 1936   | Do.                       | 23rd May 1936  | Do.  | .       |
| 12         | 20th April 1936   | Do.                       | 23rd May 1936  | Died after three days due to other causes. | .       |
| 58         | 13th June 1936  | Do.                       | 13th July 1936   | Reacted                                    | .       |
| 59         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 60         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 61         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 62         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 64         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 65         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 66         | 13th June 1936  | Do.                       | 13th July 1936   | No reaction                                | .       |
| 67         | 13th June 1936  | Do.                       | 13th July 1936   | Reacted                                    | .       |
| 68         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 69         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 70         | 13th June 1936  | Do.                       | 13th July 1936   | Do.  | .       |
| 71         | 13th June 1936  | Do.                       | 13th July 1936   | No reaction                                | .       |
| 72         | 13th June 1936  | Do.                       | 13th July 1936   | Reacted                                    | .       |

TABLE IV—*concl.*

*Cross immunity test on pigeons, immune to the English strain of pigeon-pox virus, against the Indian strain of the same virus*

| Pigeon No. | Date of inoculation with the English strain of pigeon-pox virus | Result of the inoculation | Date of cross immunity test with the Indian strain of pigeon-pox virus | Result of the cross immunity test | Remarks   |
|------------|---|---------------------------|--|-----------------------------------|-----------|
| 73         | 13th June 1936  | Reacted                   | 13th July 1936   | Reacted                           |           |
| 75         | 13th June 1936  | Do.                       | 13th July 1936   | Do.                               |           |
| 76         | 13th June 1936  | Do.                       | 13th July 1936   | Do.                               |           |
| 81         | ..  | ..                        | 13th July 1936   | Do.                               | (Control) |
| 80         | ..  | ..                        | 13th July 1936   | Do.                               | Do.       |
| 79         | ..  | ..                        | 13th July 1936   | Do.                               | Do.       |
| 78         | ..  | ..                        | 13th July 1936   | Do.                               | Do.       |

(b) *Testing the immunity of pigeons, immunized with the Indian strain of pigeon-pox virus, against the English strain of the same virus.*—Five pigeons immune to the Indian strain of pigeon-pox virus were tested with the English strain of the same virus on 6th May 1936. Pigeon No. 3 which reacted to the Indian strain resisted the English strain. Pigeon No. 2 did not react to either of the two strains. Pigeons Nos. 1, 4 and 5 reacted typically to the English strain although they were immune to the Indian strain (Table V).

In order to confirm the immunological differences between the two strains, ten more pigeons, that were previously inoculated with the Indian strain (five with filtered and the other five with unfiltered virus emulsion) were submitted to cross immunity test with the English strain of pigeon-pox virus on 26th May 1936. Of these, four pigeons showed evidence of cross immunity while the remaining six pigeons reacted to the cross immunity test which suggests some immunological difference between the two strains, although clinically both were indistinguishable (Table V).

Nine more pigeons after experimental infection with the Indian strain were tested for immunity against the English strain on 13th July 1936 (Table V). Of these, five pigeons showed no evidence of cross immunity while three pigeons had developed protection against the English strain. Pigeon No. 54 was naturally immune as it did not react to either of the two strains.

Although there was evidence of cross immunity between the two strains of pigeon-pox in some cases, yet the instances were not many, as compared with those cases where there was no evidence of cross immunity, to confirm the immunological identity of the English and the Indian strains of pigeon-pox.

TABLE V

*Cross immunity test of pigeons, immune to the Indian strain pigeon-pox virus, against the English strain of the same virus*

| Pigeon No. | Date of inoculation with the Indian strain of pigeon-pox virus | Result of inoculation | Date of cross immunity test with the English strain of pigeon-pox virus | Result of the cross immunity test | Remarks                   |
|------------|--|-----------------------|---|-----------------------------------|---------------------------|
| 1          | 20th April 1936 (with unfiltered material).                    | No reaction .         | 6th May 1936 .  | Reacted .                         |                           |
| 2          | Do. .  | Do. .                 | 6th May 1936 .  | No reaction .                     |                           |
| 3          | Do. .  | Reacted .             | 6th May 1936 .  | Do. .                             |                           |
| 4          | Do. .  | No reaction .         | 6th May 1936 .  | Reacted .                         |                           |
| 5          | Do. .  | Do. .                 | 6th May 1936 .  | Do. .                             |                           |
| 36         | 12th May 1936 (with Berkefeld 'V' filtrate).                   | Reacted .             | 26th May 1936   | No reaction .                     |                           |
| 37         | Do. .  | Do. .                 | 26th May 1936   | Reacted .                         |                           |
| 38         | Do. .  | No reaction .         | 26th May 1936   | Do. .                             |                           |
| 39         | Do. .  | Reacted .             | 26th May 1936   | Do. .                             |                           |
| 40         | Do. .  | Do. .                 | 26th May 1936   | No reaction .                     | Died due to other causes. |
| 41         | 12th May 1936 (with unfiltered material).                      | Do. .                 | 26th May 1936   | Reacted .                         |                           |
| 42         | Do. .  | Do. .                 | 26th May 1936   | Do. .                             |                           |
| 43         | Do. .  | Do. .                 | 26th May 1936   | No reaction .                     | Died due to other causes. |
| 44         | Do. .  | Do. .                 | 26th May 1936   | Do. .                             |                           |
| 45         | Do. .  | No reaction .         | 26th May 1936   | Reacted .                         |                           |

TABLE V—*contd.*

*Cross immunity test on pigeons, immune to the Indian strain pigeon-pox virus, against the English strain of the same virus*

| Pigeon No. | Date of inoculation with the Indian strain of pigeon-pox virus | Result of inoculation | Date of cross immunity test with the English strain of pigeon-pox virus | Result of the cross immunity test | Remarks                   |
|------------|--|-----------------------|---|-----------------------------------|---------------------------|
| 47         | 13th June 1936 (with unfiltered material).                     | Reacted .             | 13th July 1936  | No reaction .                     |                           |
| 48         | Do. .  | Do. .                 | 13th July 1936  | Reacted .                         |                           |
| 49         | Do. .  | Do. .                 | 13th July 1936  | Do. .                             |                           |
| 50         | Do. .  | Do. .                 | 13th July 1936  | Do. .                             |                           |
| 51         | Do. .  | Do. .                 | 13th July 1936  | Do. .                             | Died.                     |
| 52         | Do. .  | Do. .                 | 13th July 1936  | No reaction .                     | Died due to other causes. |
| 54         | Do. .  | No reaction .         | 13th July 1936  | Do. .                             |                           |
| 55         | Do. .  | Do. .                 | 13th July 1936  | Reacted .                         |                           |
| 56         | Do. .  | Reacted .             | 13th July 1936  | No reaction .                     |                           |
| 86         | .. ..  | .. ..                 | 13th July 1936  | Do. .                             | (Control).                |
| 85         | .. ..  | .. ..                 | 13th July 1936  | Reacted .                         | Do.                       |
| 84         | .. ..  | .. ..                 | 13th July 1936  | Do. .                             | Do.                       |
| 83         | .. ..  | .. ..                 | 13th July 1936  | Do. .                             | Do.                       |
| 82         | .. ..  | .. ..                 | 13th July 1936  | Do. .                             | Do.                       |

## COMPLEMENT FIXATION TESTS

Sera from natural cases of pigeon-pox and pigeons after artificial infection with the English strain were tested for the presence of complement fixing bodies. The tests were controlled by healthy sera. Kolmer's [1931] technique of the "Wassermann test" was employed with slight modifications as required. The results are tabulated in Table VI.

TABLE VI

*Complement fixation tests*

| Kind of serum             | Quantity of serum | Result with antigens |                |
|---------------------------|-------------------|----------------------|----------------|
|                           |                   | Indian strain        | English strain |
| Anti-pigeon pox (Indian)  | 0·1               | +                    | —              |
|                           | 0·05              | +                    | —              |
|                           | 0·01              | —                    | —              |
|                           | 0·005             | —                    | —              |
| Anti-pigeon pox (English) | 0·1               | —                    | —              |
|                           | 0·05              | —                    | —              |
|                           | 0·01              | —                    | —              |
|                           | 0·005             | —                    | —              |
| Healthy pigeon            | 0·1               | —                    | —              |
|                           | 0·05              | —                    | —              |
|                           | 0·01              | —                    | —              |
|                           | 0·005             | —                    | —              |
| Healthy fowl              | 0·1               | —                    | —              |
|                           | 0·05              | —                    | —              |
|                           | 0·01              | —                    | —              |
|                           | 0·005             | —                    | —              |

N.B. + = Fixation of complement (no haemolysis).  
— = No fixation of complement (haemolysis).

From the above table it appears that the complement fixing bodies are not, as a rule, demonstrable in the serum of pigeons recovered from pigeon-pox. Hence this test cannot be applied with advantage in this disease caused by either the English or the Indian strain of the virus.

## SUMMARY

A natural outbreak of pigeon-pox occurred in a stock of healthy pigeons at the Imperial Veterinary Research Institute, Mukteswar and the source of infection was not traceable. The causative agent was demonstrated to be a filterable virus and has been maintained by passage through pigeons. This appears to be the first recorded outbreak in India.

Immunological tests of the virus isolated, indicate certain degree of antigenic variation from the English strain of pigeon-pox virus although both are similar on grounds of pathogenicity and their capability to protect fowls against fowl-pox.

Preliminary complement fixation tests showed that complement fixing bodies were rarely demonstrable in the sera of healthy as well as pigeon-pox immune birds.

## ACKNOWLEDGMENTS

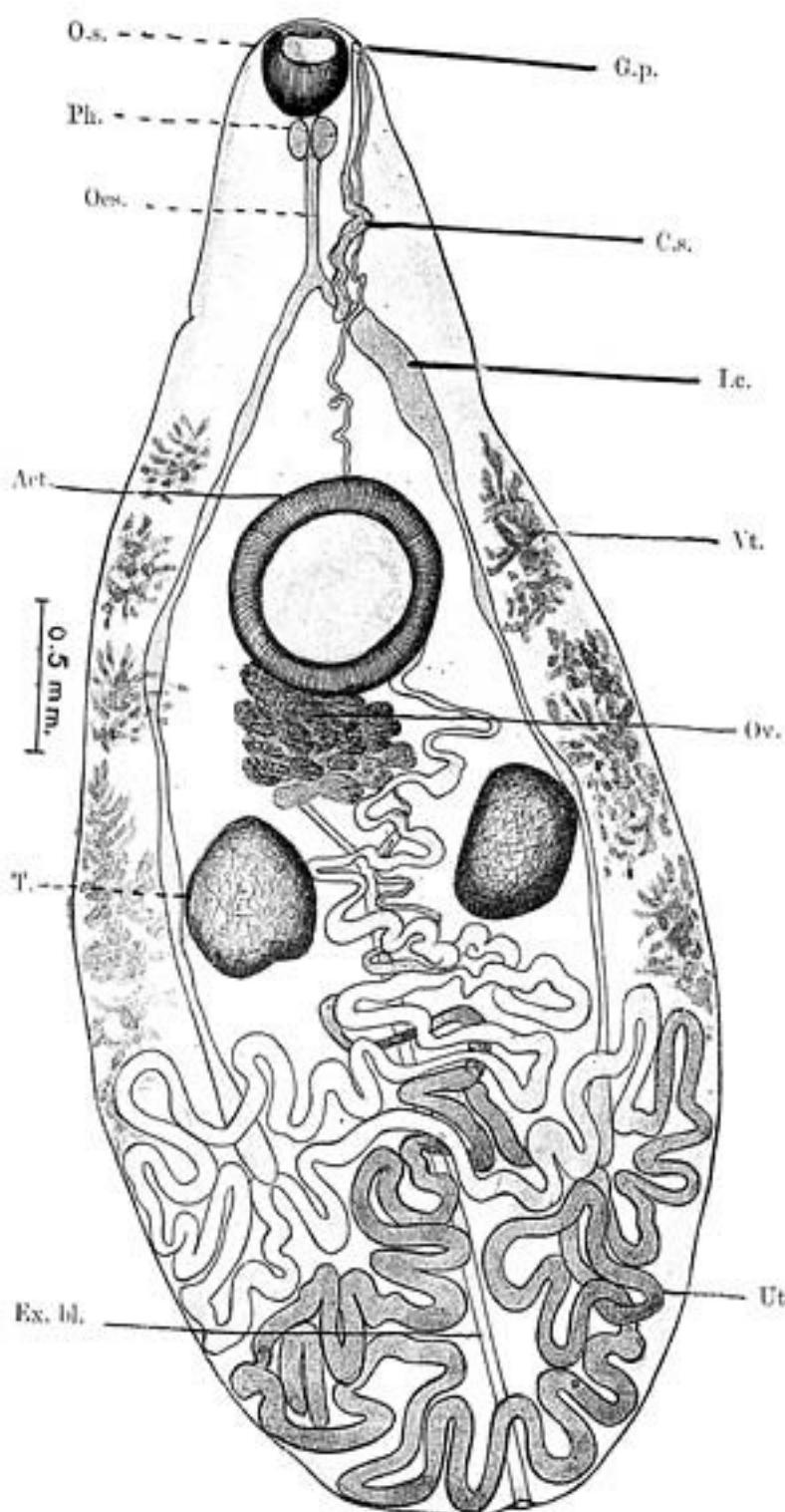
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Ventral view of *Prosthomianus indicus*, n. sp.

KEY TO LETTERING.

|         |    |                               |
|---------|----|-------------------------------|
| Act.    | .. | Acetabulum or ventral sucker. |
| C.s.    | .. | Cirrus sac.                   |
| Ex. bl. | .. | Excretory bladder.            |
| G.p.    | .. | Genital pore.                 |
| I.c.    | .. | Intestinal caecum.            |
| Oea.    | .. | Oesophagus.                   |
| Ov.     | .. | Ovary.                        |
| O.s.    | .. | Oral sucker.                  |
| Ph.     | .. | Pharynx.                      |
| T.      | .. | Testis.                       |
| Ut.     | .. | Uterus.                       |
| Vt.     | .. | Vitellaria.                   |

## SYNTHETIC POLY(URIDYLIC ACID)

| NAME   | STRUCTURE | REFERENCES |
|--|-----------|------------|
| Uridylate  |           | 1          |
| 2'-O-Methyluridylate   |           | 2          |
| 3'-O-Methyluridylate   |           | 3          |
| 4'-O-Methyluridylate   |           | 4          |
| 5'-O-Methyluridylate   |           | 5          |
| 2',3'-Dideoxyuridylate   |           | 6          |
| 2',3'-Dihydroxyuridylate   |           | 7          |
| 2',3'-Dihydro-2',3'-dihydroxyuridylate   |           | 8          |
| 2',3'-Dihydro-2',3'-dihydroxy-4'-methyluridylate   |           | 9          |
| 2',3'-Dihydro-2',3'-dihydroxy-4'-methyl-5'-methyluridylate                                     |           | 10         |
| 2',3'-Dihydro-2',3'-dihydroxy-4'-methyl-5'-methyl-2'-O-methyluridylate                         |           | 11         |
| 2',3'-Dihydro-2',3'-dihydroxy-4'-methyl-5'-methyl-2',3'-dihydroxyuridylate                     |           | 12         |
| 2',3'-Dihydro-2',3'-dihydroxy-4'-methyl-5'-methyl-2',3'-dihydroxy-4'-methyluridylate           |           | 13         |
| 2',3'-Dihydro-2',3'-dihydroxy-4'-methyl-5'-methyl-2',3'-dihydroxy-4'-methyl-5'-methyluridylate |           | 14         |

A NEW  
TREMATODE—*PROSTHOGONIMUS INDICUS*, N. SP.—  
OCCURRING IN THE OVIDUCT OF INDIAN FOWLS,  
WITH REMARKS ON 'PROSTHOGONIMIASIS'

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(With Plates XI and XII)

THE members of the genus *Prosthogonimus* are considered to be the most pathogenic trematodes of poultry. The genus was created by Luhe in 1899 with Rudolphi's *ovatus* as the type species. Two specimens of this species were first recovered by Hanow in 1753 from a freshly laid hen's egg. According to de Blieck and van Heesbergen [1923] the earliest record of the occurrence of this species is by Hanow in 1749. Subsequently a number of species has been described under the genus. At present representatives of the genus are known to occur in Europe, Asia, Africa and North and South America. The genus is now definitely assigned to the sub-family Prosthogoniminae Luhe [1909], and has been reviewed by Braun [1901] and [1902], Skrjabin [1913], Shen [1931] and Macy [1934].

Through the courtesy of Mr. S. Ganapathy Iyer, the author received a number of fowls which had succumbed to Doyle's disease and Fowl-pox for parasitological examination. From the oviducts of two fowls three specimens of a new species of the genus were recovered. One of the birds was heavily infested with *Amoebotaenia sphenoides* also.

*PROSTHOGONIMUS INDICUS*, N. SP.

Host—*Gallus gallus domesticus*.

Habitat—Oviduct.

Locality—Mukteswar-Kumaun. (The birds were received from the plains).

In the living state the trematodes are light brown in colour and possess considerable power of contraction and expansion. The body is pear-shaped with a broadly rounded hinder end and a comparatively attenuated anterior end. The cuticle is studded with minute, somewhat backwardly directed spines of  $0.015 \times 0.004^*$  size. They are closely set in the pre-testicular part but become sparse towards the hinder end. In permanent

\*All measurements are in mm.

mounts the parasite measures 4.84—8.0 in length and 2.0—2.8 in maximum breadth which occurs across the testicular region. The cup-shaped oral sucker is slightly subterminal and measures 0.18—0.32 × 0.24—0.42 in size. It is followed by a very small prepharynx, a well-developed ovoid pharynx of 0.12—0.2 × 0.18—0.26 size and a moderately long, 0.32—0.38 and narrow oesophagus. The oesophagus bifurcates into two, fairly long intestinal caeca which end blindly just in front of the posterior fifth of body length. The acetabulum measures 0.66—1.2 in diameter and is situated at the junction of the first and middle-thirds of body length. The greater part of the acetabulum lies behind the anterior third of body.

The testes, two in number, are situated almost symmetrically, one on either side of the median line, in the intercaecal space close behind the anterior half of body length. The left testis of 0.38—0.7 × 0.38—0.74 size is situated slightly cephalad to the right testis which measures 0.6—0.64 × 0.4—0.6 in size. The cirrus sac is a narrow, elongated, somewhat sinuous tube extending from the genital atrium to a little beyond the intestinal bifurcation upto the end of the anterior fifth of body length. It lies on the left side of the oesophagus and encloses an elongated, narrow, coiled vesicula seminalis which communicates anteriorly through a narrow duct with a feebly developed cirrus. The small and shallow genital atrium enclosing the male and female openings is situated on the left side in level with the anterior margin of the oral sucker.

The ovarian mass, 0.4—0.74 × 0.58—1.0 in size, consists of a large number of closely aggregated, elongated oval follicles situated almost in the median line at the middle of the body length. The greater part of the ovarian mass is pre-equatorial and slightly overlaps the acetabulum anteriorly. An inconspicuous elongated and narrow receptaculum seminis, 0.13—0.26 × 0.06—0.08 in size, lies behind the ovarian mass, a little to the left of the median line. The receptaculum seminis after giving off a small Laurer's canal opens into the oviduct. The shell gland complex is rather diffuse and is post-ovarian. The vitellaria consist of a large number of elongated oval follicles arranged laterally in seven to nine bunches. They begin from a little in front of the acetabulum and end slightly in front of the blind ends of the intestinal caeca. The vitellaria are mostly confined to the space between the intestinal caeca and the body wall. The uterus consists of coiled ascending and descending arms and occupies nearly the whole of the post-testicular space. In front of the ovary the ascending arm of the uterus runs in a more or less straight course and lies terminally to the left of the cirrus sac. It opens distally into the genital atrium independent of the male opening. The uterus is full of yellowish brown, small oval eggs of 0.019—0.021 × 0.011—0.015 size.

The excretory bladder is Y-shaped, with the main stem bifurcating into lateral cornua in the testicular region. The excretory pore lies terminally at the hinder end.

The genus contains a large number of species—*Prosthogonimus ovatus* [Rudolphi, 1803] *P. cuneatus* [Rudolphi, 1809], *P. pellucidus* [von Linstow, 1873], *P. japonicus* [Braun, 1901], *P. anatinus* [Markow, 1902], *P. putschkevskii* [Skrjabin, 1913], *P. dogiele* [Skrjabin, 1914], *P. vitellatus* [Nicoll, 1915], *P. rudolphi* [Skrjabin, 1919], *P. brauni* [Skrjabin, 1919], *P. skrjabini* [Zakharow, 1920], *P. longus-morbificans* [Seifried, 1923], *P. furcifer* [Railliet, 1924], *P. fuelleborni* [Skrjabin and Massino, 1925], *P. horiuchii* [Morishita and Tsuchimochi 1925], *P. karausiaki* [Laymann, 1926], *P. orientalis* [Yamaguti, 1933], *P. querquedulae* [Yamaguti, 1933], *P. macrorchis* [Macy, 1934], *P. leei* [Hsu, 1935]. In the proceedings of the Indian Science Congress, 1929, Gideon published an abstract of a paper on a new species of the genus *Prymnopriion* which he had obtained from the rectum of *Ibis melanocephala*. The genus *Prymnopriion* has now been proved to be synonymous with *Prosthogonimus*. The full paper has not yet been published and in the abstract the author has given no description whatsoever of the parasite, which is, therefore, considered as 'species inquizendae'.

On account of the character of its vitellaria which are arranged in definite groups and the absence of heavy uterine coils from the pre-acetabular region the new species, *P. indicus*, is referable to the sub-genus *Macrognathotrema* [Skrjabin and Baskakov, 1925]. By its above mentioned characters it can be easily distinguished from *P. ovatus* and *P. dogiele*. From *P. macrorchis* the Indian species can be distinguished by the length of the oesophagus and intestinal caeca, size ratio of suckers, position of testes and vitellaria and by the absence of the spine in the egg. In having the acetabulum more than twice the size of the oral sucker the new species can be separated from *P. furcifer*, *P. japonicus* and *P. pellucidus* in which the suckers are approximately of equal size. *P. indicus* differs from *P. vitellatus* in the size ratio of suckers and in the extent of vitellaria and the position of testes which are confined to the anterior half of body in the latter species. In the posterior extent of its cirrus sac which does not reach the ventral sucker and ends far in front of it, the Indian species differs from *P. brauni* and *P. putschkevskii*. In the post-ovarian position of testes and the pre-acetabular extent of vitellaria, the new species differs from *P. skrjabini* and *P. karausiaki* in which the testes lie more or less in level with the ovary and the vitellaria do not reach the anterior border of the acetabulum. The definitely post-acetabular character of the vitellaria in *P. anatinus*, *P. horiuchii* and *P. orientalis* distinguishes them from the Indian representative in which the vitellaria extend to a little distance in front of the acetabulum. In *P. cuneatus* the acetabulum is just twice the size of the oral sucker and the vitellaria, which do not extend in front of the acetabulum, are not divided into bunches, while in *P. indicus* the acetabulum is more than twice the size of the oral sucker and the vitellaria, which extend in front of the acetabulum, are divided into distinct bunches about seven to nine in number. *P. rudolphi* can be distinguished from the Indian form by the size of its acetabulum which is approximately twice the size of the oral sucker, the intercaecal character of its uterus and the anterior extent

of its vitellaria which do not extend in front of the acetabulum. *P. fuelleborni* differs from *P. indicus* in the much smaller size of the body ( $2.28 \times 1.17$  mm.) and the anterior extent of vitellaria which do not reach even the middle of acetabulum. *P. longus-morbificans* differs from the new species in the relative size of the acetabulum, which is one and a half times the size of the oral sucker, and the posterior extent of the cirrus sac which reaches the acetabulum. From *P. querquedulae* our species can be easily distinguished by the posterior extent of the cirrus sac, which stops far in front of the acetabulum, the topography of gonads, length of excretory bladder and the extent of vitellaria. In the size ratio of its suckers (oral sucker  $0.63 \times 0.73$ ; acetabulum  $0.87$  in diameter), extremely small length of its oesophagus, posterior extent of cirrus sac, which reaches the acetabulum, and the post-acetabular and the restricted extent of the vitellaria *P. leei* differs remarkably from the species described in this paper.

#### PROSTHOGONIMIASIS

This trematode disease, which is caused by the infestation with members of the genus *Prosthogonimus*, offers a poultry problem of the highest magnitude specially in low-lying and water-logged areas. It occurs in a large number of different species of birds and affects only the female. Amongst the domestic birds the disease has been reported from fowls, turkeys, geese and ducks. Though its effects have been observed for centuries by farmers in Europe, who were well acquainted with the symptoms and had even associated them with the extensive flights of dragonflies in certain seasons and in spite of the finding of these flukes in small numbers in the oviducts of diseased birds, no pathological significance, as has been the case with several other helminthic diseases, was attached to the presence of these parasites. In 1920, Thienemann observed the correlation between the extensive flights of the dragonfly—*Libellula quadrimaculata* and the production of soft-shelled eggs by domestic hens. He, however, attributed the disease to the presence of some toxic substance in the body of the dragonfly. In the following year Hieronymi and Szidat definitely attributed the disease to the presence of flukes—*P. pellucidus* von Linstow [Syn. *P. intercalanus* Szidat, 1921], in the oviduct and described, in detail, its symptoms and pathology.

Subsequently several new species of the genus *Prosthogonimus* have been described and a large number of cases of prosthogonimiasis have been recorded. Szidat [1927], reports that during the spring of 1926 it was impossible to obtain fresh eggs in the vicinity of Rossiten, East Prussia, owing to the devastating effects of prosthogonimiasis, and if a hen harboured more than forty or fifty parasites it usually died. The same author [Szidat, 1933], refers to this trouble as the most important disease of the low-lands of the Northern Hemisphere. Similar cases have been reported from various parts of Germany by Rheinhardt [1922], Ariess [1922], Seegart [1923], Mass [1923], and others and by de Bieck and van Heesbergen, [1923], from Holland where *P. pellucidus* has been found to be the cause. Panisset, [1924], reported similar cases of the disease from France. Kotlan and Chandler [1925] discovered that a

serious poultry disease in the United States of America was caused by a species of the genus *Prosthogonimus*. The species has since been described as *P. macrorchis* by Macy [1934], who has worked out the complete life-history of the parasite. He has extended the work of the previous American workers and has shown that the infestation is widely prevalent in the United States. Taylor [1931] pointed out that though there was no record of the occurrence of this disease in Great Britain it was likely to make its appearance sooner or later as members of the genus *Prosthogonimus* as well as the dragonfly, *L. quadrimaculata*, were known to occur in the country.

The early symptoms of prosthogonimiasis are the laying of soft-shelled eggs and a marked decrease in the egg production. To determine the effect of the infestation with *P. macrorchis*, Macy [1934], carried out experiments on laying hens. He selected a flock of sixteen white-leghorn hens with known laying records and to eight of them he fed cysts of the parasite obtained from naïads, the other eight birds were kept as controls. During a fixed period of observation he found that the controls laid ten times as many eggs as did the infested birds. The striking fall in egg production was due entirely to *P. macrorchis*. Thus it would appear that the worms when present even in such small numbers as will not cause death of the bird, can be responsible for enormous economic loss to poultry farmers.

The parasite usually lives in the bursa fabricii of both male and female birds. In laying birds it enters the oviduct, being probably assisted by the movements of that organ, and is responsible for causing acute inflammation and the consequent production of abnormal eggs and discharge of albumen. Owing to irritation retroperistaltic movements are set up in the oviduct causing broken yolk, albumen, bacteria and parasite material to enter the peritoneal cavity and giving rise to acute peritonitis. The disease is frequently fatal in even moderately heavy infestations. An interesting, though not serious, aspect of the disease is the occasional occurrence of living parasites and parasite material inside the shell of otherwise normal eggs. This inclusion is possible because the parasite lives on the surface of the oviduct which secretes the egg material.

Kolan and Chandler, [1925], noticed the following pathological changes in diseased birds on *post mortem* examination; heavy emaciation and anaemia; fibrinous peritonitis, with a large amount of a sticky, yellow exudate containing large masses of egg-yolk and albumen, parasites and its eggs. The ovary showed a number of diseased, collapsed ovules, containing greyish-yellow, egg-yolk-like material mixed with fibrin and pus. The oviduct was greatly distended, its serous coverings showing a more or less pronounced reddish discolouration. The lumen of the oviduct contained a large amount of albumen material forming ovoid clots of about one to two cm. in diameter; the mucosa was covered with a sticky exudate consisting mainly of albumen, blood and fibrin; large number of flukes was also present. Bacteriological examinations, both microscopical and cultural, were negative. Bittner [1923], and Macy [1934], found infiltration of large numbers of plasma cells

in the tissues of the diseased oviduct. The former author found large numbers of eosinophiles also but Seifried [1923] and Macy [1934] report the presence of relatively few eosinophiles.

The diagnosis of the disease is usually difficult on account of its obscure symptoms, specially in the initial stages. The earlier symptoms, laying of soft-shelled eggs and decrease in egg production, are likely to be attributed to disturbances of the oviduct arising from nutritional irregularities. A positive diagnosis is possible only by the finding of mature parasite or its eggs in the faeces or in the eggs of the suspected bird. It is, however, not always possible to determine the infestation by faecal examination. Seifried [1923], Macy [1934], and others have reported their inability to find eggs in the faeces of infested birds, specially when the infestation was not very heavy, but enough to cause trouble. In the absence of a constant passage of bulky material through the oviduct, the eggs are probably discharged only periodically. In the absence of a positive diagnosis, the following symptoms, when taken collectively, are strongly suggestive of prosthogonimiasis. Inactivity of the bird, sharp decline in egg-production, laying of soft-shelled eggs, presence of chalky-white crust on the feathers around cloacal region, distension of the abdomen in the cloacal region and bluish-red colour of the skin of the abdomen (advanced cases), presence of hard, cream-coloured chunks of abortive egg-white and yolk in the oviduct and abdominal cavity and the distended oviduct filled with fibrinated pus and abdominal cavity with pus-exudate, parasites, eggs, lesions and peritonitis.

The worms in the oviduct can not be removed by any method of treatment. In the early stages, while the parasites are still in the intestine, it may be possible to remove them and thus prevent their entry into the oviduct. Bunyea, Hall and Cram [1933], have reported successful results in the treatment of prosthogonimiasis with carbon tetrachloride when given in repeated doses of 1.5 to 1.7 c.c. in liquid cereal. On account of its toxicity the drug should be used with care.

The occurrence of the parasite in migratory birds and the lack of marked host specificity and the migratory nature of its second intermediate host render the problem of prevention extremely difficult. The author understands from Dr. Mehra of the Allahabad University that the Indian snipes are commonly infested with members of the genus *Prosthogonimus*. Though the life-history of the Indian species has not yet been worked out, from a comparative study of the life-histories of other species of the genus, it is highly probable that the infestation of poultry with this parasite in this country, as elsewhere, takes place through the young and the adult of dragonflies. The only possible method of preventing the infestation is by keeping poultry fenced away from permanent ponds and lakes. Adult dragonflies are easily caught by poultry on damp mornings and hence the birds should not be let loose in the mornings until the dragonflies are out of the weeds.

The author is deeply grateful to the Director and the Pathologist for their kind encouragement and help.

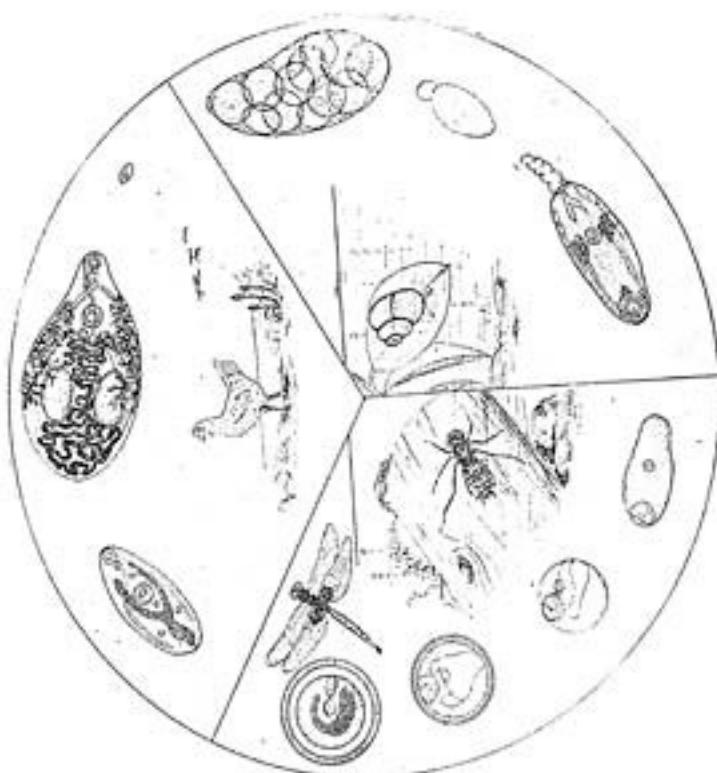


FIG. 2. Life cycle of *P. marporhe* (after Muzy, 1934)

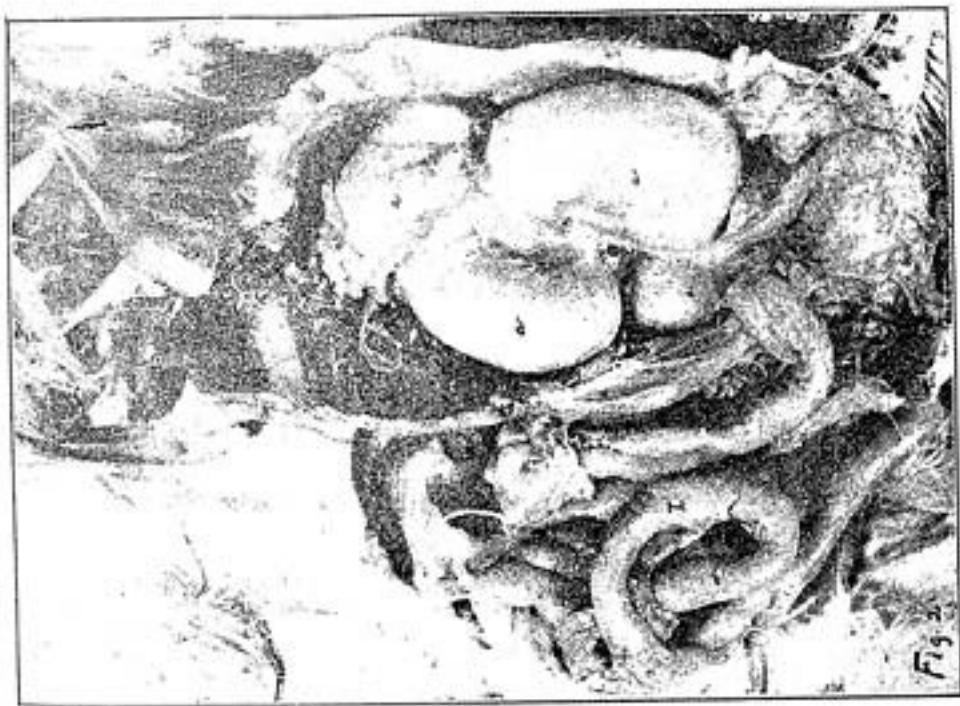


FIG. 3.

FIG. 1. Internal organs of chicken infested with *Prosthospermae* species. (after Kotlan and Chandler, 1925). *a*, albuminous clots in oviduct; *b*, albuminous clot in peritoneal cavity; *c*, intestines; *d*, heart.



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FIG. 1.

Section of growth showing large numbers of sporangia underlying the epithelium



FIG. 2.

Sporangia in various stages of development as seen under low power



FIG. 3.

An immature sporangium with a thick capsule as seen under high power

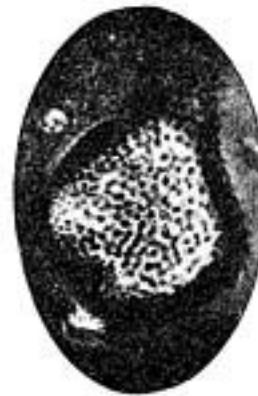


FIG. 4.

A mature sporangium with fully-developed spores as seen under high power

## RHINOSPORIDIOSIS IN EQUINES

BY

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(With Plates XIII and XIV)

THE first published reference to the occurrence of Rhinosporidiosis in animals in this country is to be found in a paper entitled "Rhinosporidiosis in Cattle" read by the late Krishnamurti Ayyar at the seventh Congress of the Far Eastern Association of Tropical Medicine held in Calcutta in 1927, wherein after reviewing the literature on the subject, he recorded the discovery of the parasite in growths from the nasal cavities of two bullocks and one cow in the Tanjore district of Madras. Up till now this has been the only record of the occurrence of the disease in bullocks either in this country or outside.

Later, in 1932, Ayyar described Rhinosporidial growths in a mare which also came from the Tanjore district. Though this was the first case of its kind to be recorded in a horse in India, the parasite had been described earlier in a horse, by Zschokke [1913] in growths sent to him from South Africa. The growth from the nasal cavity in which the parasite was found was of the size of an egg, reddish in colour and hard with a rough outer surface and inside it were found cysts which were filled with a parasite which was very closely allied to *Rhinosporidium seeberi*, the parasite found in man and which Zschokke termed *Rhinosporidium equi*.

In 1926, two further cases were recorded in the same country in mules by Quinlan and de Koch, and in 1928 another in a horse in Montevideo by Cordero and Vogeslang, the lesions in all these cases being more or less alike and in the nature of irregular tumour-like masses situated on the nasal mucous membrane.

Including the case recorded by Krishnamurti Ayyar, there are on record only five cases of Rhinosporidiosis in equines. The object of this small note is to place on record another case of equine Rhinosporidiosis in a country-bred pony at Bargarh in the Sambalpur district of Orissa.

During the author's visit to Bargarh in February, 1937, Veterinary Assistant Surgeon Y. K. Viswanathan, in-charge of the local Veterinary Dispensary, showed him a small growth which he had removed from the nasal cavity of a horse. Suspecting that it might be a Rhinosporidial growth, the writer brought it to Patna where the diagnosis was confirmed by histological examination.

The following are the available details about the animal :—

|                                     |  |
|-------------------------------------|--|
| District . . . . .                  | Sambalpur.   |
| Sub-division . . . . .              | Bargarh.   |
| Village . . . . .                   | Bargarh.   |
| Breed and sex . . . . .             | Country-bred chestnut mare.                                      |
| Age . . . . .                       | 14 years.  |
| Nature and size of growth . . . . . | Soft about 1" long and $\frac{1}{2}$ " high.                     |
| Symptoms . . . . .                  | Slight blood-stained mucous discharge from the affected nostril. |
| Date when first seen . . . . .      | 14th February, 1937.   |
| Date when growth removed . . . . .  | 15th February, 1937.   |
| Subsequent history . . . . .        | Not known, the animal being sold off.                            |

The animal was brought to the hospital as it had audible breathing and there was a slight bloodstained mucus discharge from one of the nostrils. The Veterinary Assistant Surgeon while examining the affected nostril found a small growth in the anterior part of the nasal cavity and he removed it.

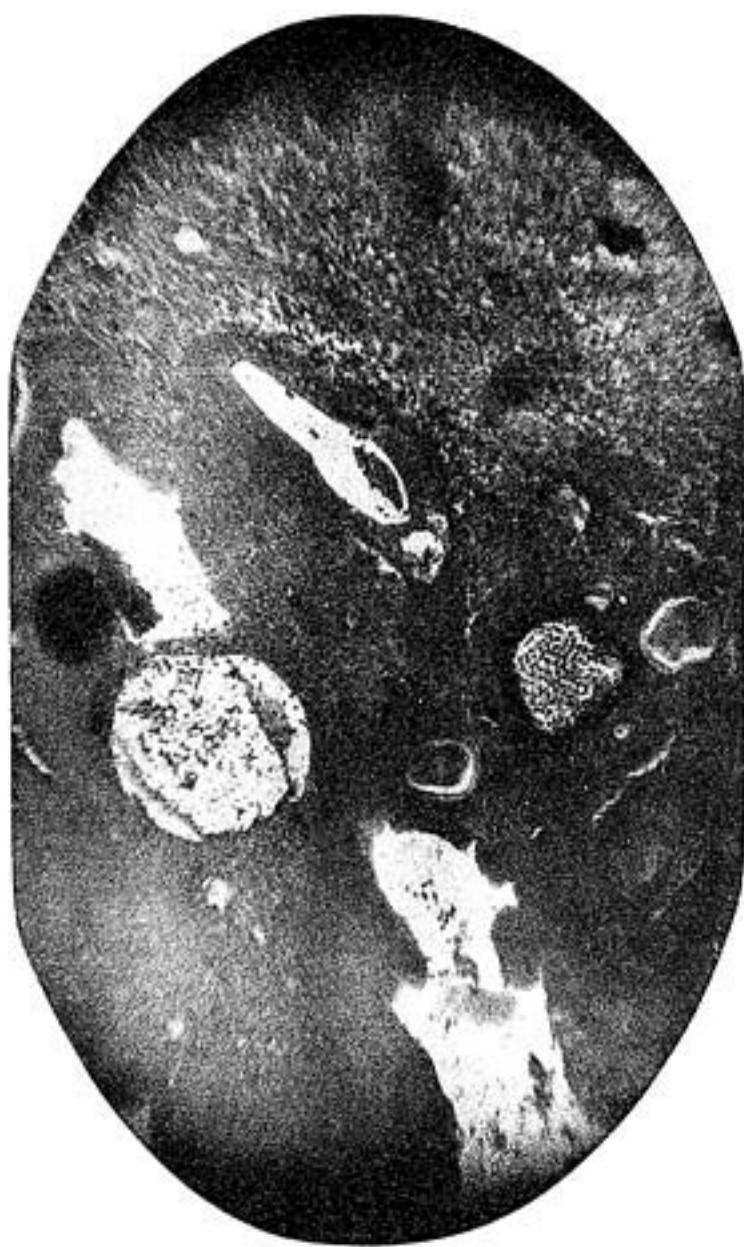
The growth was about one inch long and half inch high and on naked eye examination, it presented a cauli-flower-like appearance with a soft irregular surface. When examined under a hand lens, it presented numerous pale white specks embedded in soft tissue, which on histological examination proved to be cysts or sporangia of *Rhinosporidium*.

Histologically the growth appeared to be identical in every respect with the one described by Krishnamurti Ayyar. It was covered with stratified squamous epithelium which presented an irregular outline and was in places thrown into folds. In the superficial layers of the underlying highly vascular sub-epithelial connective tissue were present large numbers of sporangia in various stages of development. They were either oval or spheroidal in outline and each was enclosed in a capsule which was thicker in the young than in the older sporangia.

Inside each sporangium were present numerous spores in various stages of development, the fully formed ones being usually in the centre and the small ones at the periphery. In a few the fully-formed ones were situated at one pole and the small ones at the other. The spores were enclosed in a thick chitinous envelope with granular bodies inside them. Those bodies are considered by some to be refrangent spherules and by others to be sporules or sporozoites.

As the sporangia grew older, they increased in size and ultimately pressed upon the overlying epithelium which gradually diminished in thickness and ultimately burst along with the capsules of the sporangia, thus enabling the spores to get discharged on the surface of the nasal mucous membrane.

The case described in this paper is the second of its kind to be discovered in a horse in India. Careful enquiries failed to reveal the existence of more cases in the locality.



A mature sporangium rupturing to discharge the spores with other  
sporangia in various stages of development.



In view of the close relationship between *Rhinosporidium equi* and *Rhinosporidium seeberi*, the causal organism of Rhinosporidiosis in man, enquiries were made to ascertain if human Rhinosporidiosis had been recorded in the locality but the author learnt that the Medical Department was not aware of the existence of the disease in Bihar. There is, however, a record of one case of human Rhinosporidiosis in the Patna General Medical Hospital and two in the Cuttack district of Orissa. In view of the fact that the disease has now been shown to exist in this part of the country, besides Madras Presidency, systematic search will probably reveal the existence of the disease in other parts of India also.

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THE OCCURRENCE OF *CORYNEBACTERIUM EQUI* IN A  
SHE-BUFFALO

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(With Plate XV and one text-figure)

*CORYNEBACTERIUM EQUI* was first described by Magnusson [1923] as a possible etiological agent in specific pneumonia in foals and he recorded twelve cases of its incidence in a breeding establishment in Sweden. He also conducted experiments which indicated that only equines and pigs were susceptible to infection with the bacillus responsible for pneumonia in foals.

Subsequent workers have also reported the occurrence of the disease in equines in other countries; Meissner and Wetzel [1923], Lutje [1923], and Lund [1924] in Germany; Bull [1924] in Australia; Dimock and Edwards [1931] in America and Rajagopalan [1937] in India.

This organism has so far been recorded as occurring exclusively in the equine species. The object of this paper is to record the occurrence of an organism indistinguishable from *Corynebacterium equi* in its morphological, cultural, biochemical and pathological characters in the genital tract of a she-buffalo, the subject of abortion.

We are indebted to Mr. Tulsi Ram, Veterinary Assistant Surgeon on special duty at Okara, for the material from the she-buffalo. The material was received as swabs taken from the os uteri. The following history was supplied with the material:

"Buffalo No. 92/4-3 in first calving—aborted about eight days ago—eight months pregnancy—purchased from outside in pregnant state. Symptoms: After-birth came out normally after abortion—discharge scanty, but purulent and offensive—swab taken from vaginal wall near os uteri, as the animal was badly straining and not properly secured."

At the same time and in the same place three other she-buffaloes also aborted with the same clinical history.

As the case was suspected for bovine contagious abortion, the usual cultural examination and small animal inoculation were done. On cultural, biological and serological examinations, the material turned out to be negative for evidence of infection with *Br. abortus*. But an *Escherichia* species and a pigmented diphtheroid (*C. equi*) which is the subject of the present report, were isolated.

## THE IDENTITY OF THE ORGANISM

*Morphology.*—Morphologically the organism grown on solid medium, appears as short rods, mostly straight, occasionally slightly bent, with rounded or clubbed ends. Most of them are small, almost ovoid, approaching the spherical quite frequently. It is Gram-positive, non-motile, non-spore-forming and non-capsulated. In broth-culture the bacillary form is more pronounced than the coccoid.

*Cultural characters.*—Grown on plain agar plate, colonies are raised and circular, with entire margin and moist and shining surface. They are fawn-coloured and slimy in consistency. The colonies coalesce and tend to run down and accumulate at the bottom of the slant. If stored at the ordinary room temperature and exposed to light, after an incubation of twenty-four hours at 37°C., the colour of the growth gradually deepens, becoming salmon-pink.

In broth, growth is manifested by an uniform turbidity with a slight granular deposit at the bottom which disintegrates completely on shaking. The deposit develops a faint fawn colour after two or three days. Frequently a ring of granular growth on the surface is also noticed.

It grows aerobically as well as under micro-aerophilic conditions, but growth under anaerobic conditions, if any, was not noticeable.

The biochemical and other properties are tabulated below (Table I) along with those of *C. equi*, for comparison:—

TABLE I

| Tests     | <i>C. equi.</i> | Organism isolated | Remarks |
|-----------|-----------------|-------------------|---------|
| Adonite   | —               | —                 |         |
| Dextrose  | —               | —                 |         |
| Inulin    | —               | —                 |         |
| Maltose   | —               | —                 |         |
| Raffinose | —               | —                 |         |
| Salicin   | —               | —                 |         |
| Xylose    | —               | —                 |         |
| Arabinose | —               | —                 |         |
| Dulcite   | —               | —                 |         |

TABLE I—*contd.*

| Tests             | <i>C. equi.</i>                                | Organism isolated                              | Remarks                    |
|-------------------|--|--|----------------------------|
| Glycerol          | —  | —  |                            |
| Lactose           | —  | —  |                            |
| Mannitol          | —  | —  |                            |
| Rhamnose          | —  | —  | Readings upto eleven days. |
| Sorbitol          | —  | —  |                            |
| Galactose         | —  | —  |                            |
| Dextrin           | —  | —  |                            |
| Inositol          | —  | —  |                            |
| Laevulose         | —  | —  |                            |
| Mannose           | —  | —  |                            |
| Sucrose           | —  | —  |                            |
| Starch            | —  | —  |                            |
| Trehalose         | —  | —  |                            |
| Voges-Proskauer   | —  | —  |                            |
| Methyl red        | —  | —  |                            |
| Indol production  | —  | —  |                            |
| Nitrate reduction | +  | —  | +                          |
| Hydrogen sulphide | —  | —  |                            |
| Catalase          | +  | —  | +                          |
| Litmus milk       | No change                                      | No change                                      | 15 days                    |
| Gelatine          | No liquefaction                                | No liquefaction                                |                            |
| M. B. Reductase   | —  | —  |                            |
| Potato            | Moderate dry growth red-dish-yellow in colour. | Moderate dry growth red-dish-yellow in colour. |                            |

TABLE I—*concl.*

| Tests                              | <i>C. equi.</i>  | Organism isolated  | Remarks |
|------------------------------------|--|--|---------|
| Growth on Sodium tellurite medium. | Deep black   | Deep black.  |         |
| Loeffler's medium                  | Moderate growth less abundant than on agar.<br>Light pink. | Moderate growth less abundant than on agar.<br>Light pink. |         |
| Blood agar                         | No haemolysis  | No haemolysis.   |         |

It is, therefore, evident that the organism isolated has characters identical with those of *C. equi*.

#### SEROLOGICAL TESTS

(1) *Agglutination*.—When the material was first received, suspecting the case might be one of abortion due to infection with *Br. abortus*—the common causal organism of bovine abortion—a guinea-pig had been inoculated intra-peritoneally with the material. This was bled twenty-seven days later, and the separated serum was put to agglutination test against the organism under report as well as against *Br. abortus*. No agglutination took place in any dilution in both instances.

Two rabbits were immunised, one with *C. equi* of foal pneumonia origin and the other with the diphtheroid from the specimen. They were injected intravenously with saline emulsions at four hundred millions per c.c. of the respective organisms, three times at five days intervals. The dosage for the successive injections was 0·25, 0·5 and 1 c.c. respectively. They were bled on the eighth day after the last injection. The two sera were put to the agglutination test against their homologous organisms. At the same time they were put to cross-agglutination tests, the serum of the equine source against the buffalo-strain of diphtheroid, and vice versa. No agglutination was obtained in any of these tests. These tests were repeated in saline concentrations up to 1·5 per cent, still with negative results.

The fact, that the guinea-pig serum failed to reveal any titre for the organism isolated and the sera of rabbits specially treated for the purpose also failed to give any agglutination, is not, however, very disappointing. For, working with *C. equi* isolated from cases of foal pneumonia, we have never been able to demonstrate agglutinins in the naturally infected subjects, and only occasionally in small animals intensively treated for the production of agglutinating sera. This seems to have been the experience of most other

previous workers as well. Magnusson [1923] has reported his failure to obtain agglutination of the bacillus with the blood of sick animals and also from rabbits which had received repeated intravenous injections of the culture. Bull [1924] also failed to demonstrate agglutinins in a horse injected subcutaneously with an emulsion of the culture. Dimock and Edwards [1931] however, claim to have produced agglutinating serum (titre 100), by giving the horse four injections of culture with increasing dosage at intervals of seven days.

(2) *Complement fixation test*.—As the agglutination tests were of no avail in proving the identity of the two organisms, recourse was then had to the complement fixation test, and cross complement fixation tests.

The sera from the two immunised rabbits, already mentioned, were used as the source of amboceptors. The Kolmer's technique was followed in these tests, using two full units of complement in one c.c. volume and 0.5 c.c. quantities of each of the four other components.

The haemolytic serum was titrated using falling quantities of a 1 in 1,000 diluted serum, fixed quantities of complement, i.e., 0.3 c.c. and  $\frac{1}{2}$  c.c. quantities of a two per cent suspension of sheep red blood corpuscles. The mixture was incubated for one hour at 37°C. and the titre at which haemolysis occurred was read off. The stock haemolysin was then diluted for the test accordingly, so that 0.5 c.c. contained two units of haemolysin.

Serum for complement was obtained from a healthy guinea-pig and was diluted initially 1 in 30. Dilutions of complement from 0.1 to 0.5 c.c. with a graded increase of 0.05 c.c. were made in normal saline solution to which two units of haemolysin and two per cent suspension of sheep R. B. C., each in half c.c. quantities were added and incubated for one hour at 37°C. The smallest amount of complement giving complete haemolysis was noted and the next higher, which is the full unit was taken as a guide to prepare a dilution to contain two full units in one c.c. quantities.

Graded dilutions, in geometrical proportion, were made of the antigen, for titration, from 2 to 32. Half c.c. quantities of a 1 in 10 dilution of a heated healthy serum and one c.c. quantities of complement containing two full units were added. After two hours' incubation at 37°C., haemolysin and R. B. C. in required quantities were added followed by a further incubation of one hour at 37°C. The smallest amount of antigen producing some inhibition of haemolysis was noted and the stock antigen diluted for the test in such a way that one-third of the anti-complementary unit was contained in  $\frac{1}{2}$  c.c. volume.

Having titrated the components the complement fixation test was conducted using the sera of the two rabbits which had been immunised against *C. equi* and the buffalo diphtheroid respectively. The sera were heated overnight at 55°C. Dilutions of the serum from 1 in 10 to 1 in 800 were made, and the titrated quantities of antigen and complement were subsequently

added and incubated for two hours at 37°C. Then the requisite quantities of haemolysin and sheep R. B. C. were added. The contents were mixed well and incubated for one hour at 37°C.

A healthy rabbit serum was also tested against both the antigens for control.

In addition, a complete set of the usual controls for the reliability of the components were kept as follows:—Serum control with one-tenth dilution of serum, complement, haemolysin and R. B. C., but with no antigen; antigen control with  $\frac{1}{2}$  c.c. of antigen, complement, haemolysin and R. B. C., but with no serum; haemolytic system control with two units of haemolysin complement and R. B. C., but with neither serum nor antigen; R. B. C. control with two per cent sheep R. B. C. in normal saline only.

All controls were satisfactory.

The result is tabulated below (Table II).

TABLE II

| Serum tested (after heating at 55° C. overnight) | Serum dilutions |      |       |       |       |       | Antigen | Controls |         |                   |          |
|--|-----------------|------|-------|-------|-------|-------|---------|----------|---------|-------------------|----------|
|  | 1/10            | 1/50 | 1/100 | 1/200 | 1/400 | 1/800 |         | Serum    | Antigen | Haemolytic system | R. B. C. |
| <i>C. equi</i> of foal pneumonia origin          | ++++            | +++  | ++    | —     | —     | —     | C. 13   | —        | —       | —                 | +        |
| Do. . .  | ++++            | +++  | +     | —     | —     | —     | C. 200  | —        | —       | —                 | +        |
| Diphtheroid of buff-cow origin. . .              | +++             | ++   | +     | —     | —     | —     | C. 13   | —        | —       | —                 | +        |
| Do. . .  | +++             | ++   | +     | —     | —     | —     | C. 200  | —        | —       | —                 | +        |
| Healthy rabbit serum . . .                       | +               | —    | —     | —     | —     | —     | C. 13   | —        | —       | —                 | +        |
| Do. . .  | +               | —    | —     | —     | —     | —     | C. 200  | —        | —       | —                 | +        |

NOTE:—C. 13 = *C. equi* of foal pneumonia origin.

C. 200 = Diphtheroid of she-buffalo origin.

(—) = Complete haemolysis.

} No fixation of complement.

(+) = 25 per cent inhibition of haemolysis.

(++) = 50 per cent inhibition of haemolysis.

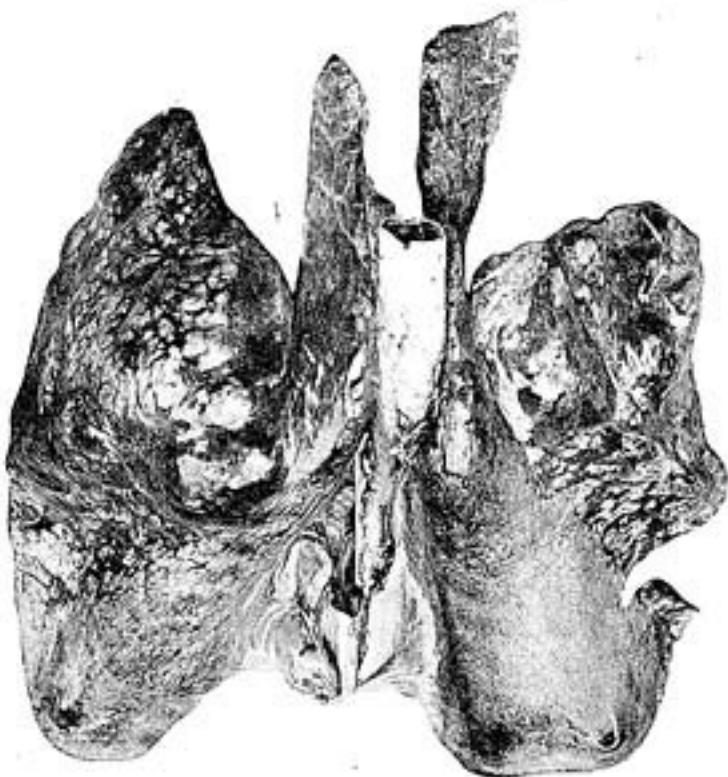
(+++) = 75 per cent inhibition of haemolysis.

(++++) = 100 per cent inhibition of haemo-

} Degrees of fixation of comple-

ment.





The lung of Bull No. 117 that was infected with the buffalo strain of diphtheroid, showing abscesses in the anterior portion of both the main lobes.

It will be seen that the foal pneumonia organism fixes complement in the presence of the buffalo-diphtheroid-serum at about the same titre as in the presence of its homologous serum and vice versa.

#### PATHOGENICITY

The organism was found to be non-pathogenic to rabbits and guinea-pigs. With a view to ascertain the pathogenicity of the organism for young equines, Foal No. 417, aged fourteen days was swabbed inside both nostrils with an emulsion of the buffalo strain of diphtheroid. The animal behaved exactly like a foal infected with *C. equi* (Text-fig. 1). It showed a distinct rise in temperature on the evening of the sixth day. The animal was dull and lacking in appetite. It had an occasional cough and became progressively weak. The respiration was distressed and gurgling noises could be heard on auscultation of the lungs. The temperature continued to be high until just before death when there was a slight fall. The foal died on the sixteenth day after infection. A *post mortem* examination, the anterior portion of both the main lobes of the lungs were found studded with characteristic abscesses (Plate XV). An organism indistinguishable from *C. equi* was re-isolated from the lung abscesses in pure culture.

Whether the organism is capable of causing abortion or purulent metritis in she-buffaloes under experimental conditions could not be determined owing to lack of pregnant she-buffaloes. In the hope that a pregnant cow might react in a similar way, Cow No. 5, nine months pregnant, was instilled intravaginally with five c.c. of a saline emulsion of the organism made from four 48-hrs. old agar slants, combined with an oral administration of an equal quantity of a similar emulsion. Twelve days later the cow gave birth to a calf which died soon after birth. The organism could not be recovered from the uterine discharges of the cow, nor from any of the internal organs of the dead calf.

Another experiment was conducted to find whether this organism could cause pneumonia or joint-ill in bull-calves, in the same way as *C. equi* does in young equines. Two calves, aged two to three months, were swabbed in both nostrils with an emulsion of the organism. No lesions attributable to infection with *C. equi* developed in either of them.

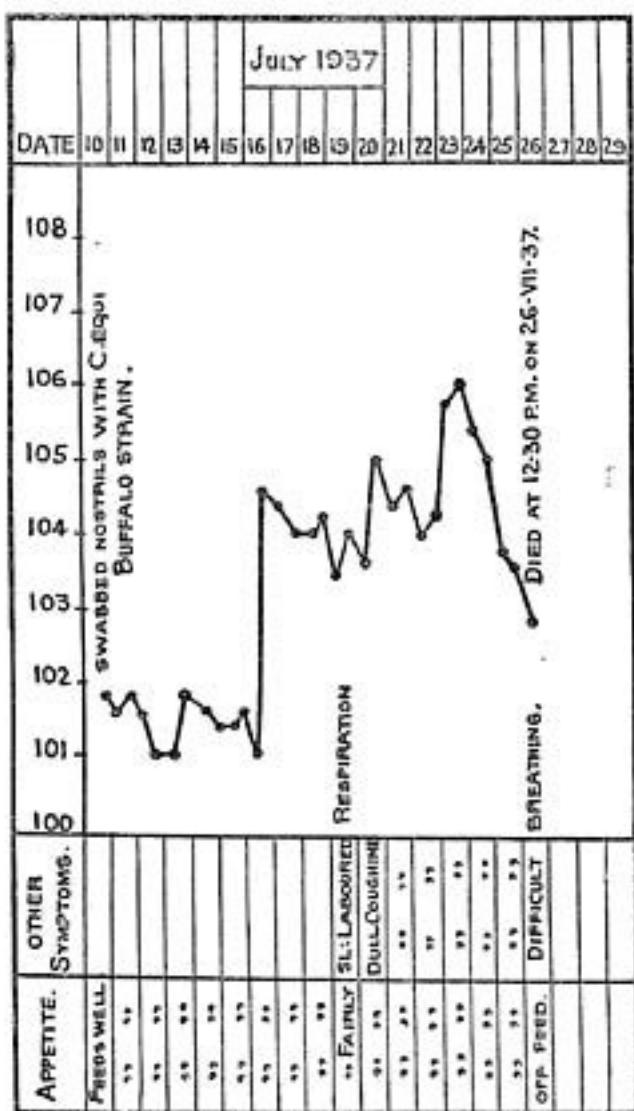


Fig. 1.

Clinical chart of Foal No. 417 (born on 26th June 1937) infected with the buffalo strain of *C. equi*.

## DISCUSSION

From the cultural and biochemical characters of the organism coupled with its serological affinity to *C. equi* and pathogenicity to young foals, there remains no doubt the organism under consideration is a strain of *C. equi*.

This is the first time that the organism has been recorded in a species other than equine. It was isolated from the uterus of a she-buffalo that had aborted. Following abortion there was a foetid purulent slimy discharge from the uterus and the organism was obtained from this discharge. The only other organism that was grown besides *C. equi* was a coliform organism and the question naturally arises why the latter should not be held as the cause of the condition. It is true that certain strains of coliform organisms are capable of causing inflammatory conditions of the urinogenital tract of man and animals. Such strains are highly virulent for the guinea-pig and mice, causing, on fresh isolation, by intraperitoneal inoculation, a suppurative or purulent peritonitis or a fatal septicaemia. But in this case, guinea-pigs and mice that had received intraperitoneal injections of the material developed no illness, and on destruction revealed no pyogenic lesions. It would appear, therefore, that the coliform organism was a banal organism devoid of pathogenic properties. There is, therefore, the probability that the pyogenic lesions met with in the buffalo were due to the other organism isolated namely *C. equi*. This organism is non-pathogenic to laboratory animals, which fact should explain why the guinea-pigs and mice inoculated with the purulent material from the buffalo containing the diphtheroid developed no lesions.

But the mere isolation of an organism from lesions does not definitely establish it as the pathogen. Lesions similar to those in the buffalo could not be induced in a pregnant cow by the intravaginal instillation of the isolated organism. Owing to lack of pregnant buffaloes, the pathogenicity of the organism to the species from which it was isolated could not be tried under experimental conditions. It has not been possible, therefore, to advance experimental evidence definitely incriminating this organism as the cause of either post-abortion purulent metritis or of abortion although the circumstances of the case under report are strongly suggestive of its being the cause of one or both. However, irrespective of its pathogenic significance, the mere occurrence, in the buffalo, of *Corynebacterium equi*, an organism which has so far been naturally met with exclusively in the equine species, is of sufficient interest and is deserving of report.

## SUMMARY

*Corynebacterium equi* has been recognised by several workers as a cause of pneumonia in young equines. It has not so far been recognised as occurring in any other species of animals. Its occurrence in pyometra following abortion in a she-buffalo is, therefore, recorded in this article. Its identity with *Corynebacterium equi* has been proved by morphological, cultural, biochemical,

serological and biological tests. The probability of its being an etiological factor of either abortion or of post-abortion pyometra is discussed.

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*PSEUDANOPLOCEPHALA CRAWFORDI* BAYLIS, 1927.

BY

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[With Plate XVI and two text-figures.]

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INCIDENCE of adult cestodes in the pig appears to be very limited. The cases on record so far are by Maplestone and Southwell [1923], Baylis [1927] and Hall [1924]. Maplestone and Southwell [1923] described *Paramoniczia suis* from an Australian wild pig. Baylis [1927] described *Paramoniczia phacocheiri* from an African wart hog and *Pseudanoplocephala crawfordi* obtained from a wild pig in Ceylon. Hall [1924] appears to have recorded an undetermined Anoplocephalid tapeworm in a pig in America. It is thus seen that no adult tapeworms have so far been reported from India and hence this record of a tapeworm from this part of India. In the last week of December 1937, a collection of helminths of the pig was made by the writers and the worm to be described belongs to that collection. In the museum of parasitological collections of this College, about half a dozen specimens of tapeworms from the pig collected by M. Anant Narayan Rao and L. S. Parameshwara Iyer during 1932 and awaiting identification were also examined. All the specimens resemble, in almost all details, the parasite *P. crawfordi* Baylis, 1927. A description of the parasites is given below :

The worms measure from 21 to 31 cm. in length and 3 to 4 mm. in width. The segments are wider than long in the anterior portion while there is a tendency to an increase in the length at the posterior end of the strobila. The gravid segments are very much contracted.

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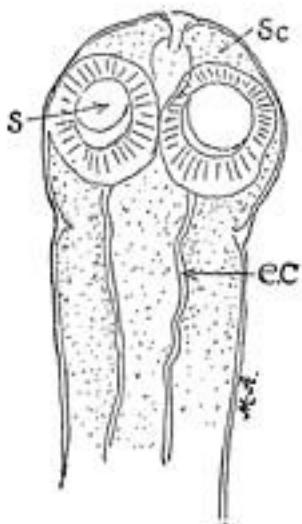


Fig. 1—Scolex of *Pseudanoplocephala crawfordi*, showing the suckers, neck and the commencement of the longitudinal excretory canals. (Sc-scolex ; S-sucker ; ec-excretory canals).

The scolex (Fig. 1) is small and measures 0·45 mm. in width. The suckers are directed upwards and forwards and vary from 0·10 to 0·14 mm. in diameter. The neck is constricted and measures 0·35 mm. in width.

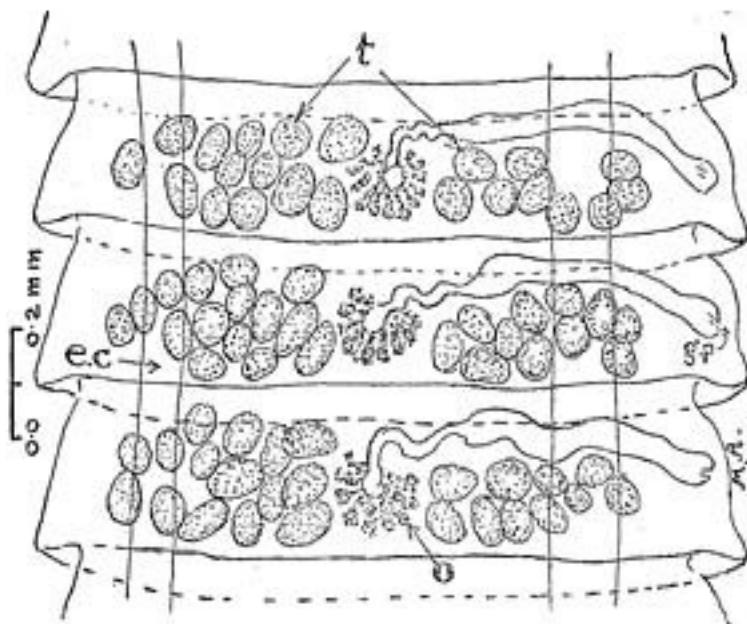
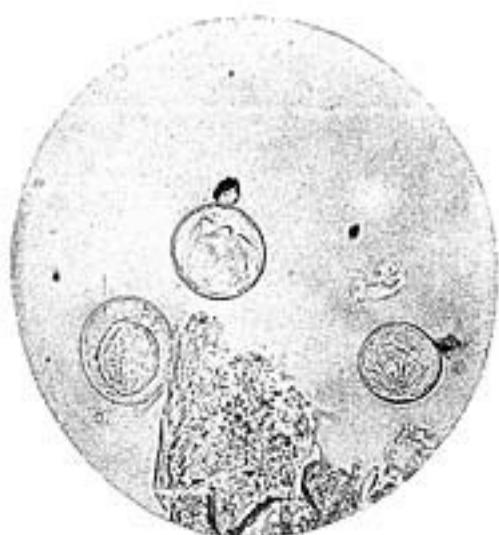


Fig. 2—A portion of the strobila showing the pair of longitudinal excretory canals and the genitalia. (t-testes ; e.c.-ovary ; g.p-genital pore.)





Microphotograph of the ova of *Pseudotaplocephala Crawfordi*  $\times 630$

The excretory system (Fig. 2) consists of a single pair of longitudinal canals only, situated ventrally with no cross communication at the posterior end of the segments. They are wide and wavy and measure 0·075 to 0·10 mm. in width.

*Genitalia*.—There is a single set of reproductive organs in each segment. A paired ovary with finger-like processes all round, a vitelline gland which is also lobed, occupy the median area of the segment. The vagina lies ventrally to the cirrus which is lodged in a well-developed pouch or sac. The cirrus is continued inwards into a fusiform vesicula seminalis into which the vas deferens merges. The testes extend across the whole segment being more on the aporal than on the poral side and vary from twenty-five to twenty-six in number. The uterus is an elongated sac with many out-pockets and contains ova (Plate XVI) measuring 0·10 mm.

#### ACKNOWLEDGMENTS

The authors are indebted to Rao Sahib M. Anant Narayan Rao for his valuable help and the drawings and to the college artist, Mr. Duraiswamy Mudaliar for the microphotograph.

#### CONCLUSIONS

An adult tape worm so far undescribed from India is recorded in this paper. This appears to be a fairly common worm infesting the intestines of domestic pigs in South India.

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STUDIES ON THE HELMINTH PARASITES OF INDIAN  
POULTRY, PART II. THE OCCURRENCE OF  
GAPEWORM IN FOWLS

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THE terms gapeworms, y-worms and forked-worms are applied to a genus of strongyle nematodes—*Syngamus* v. Siebold [1836], which occur in the adult stage in the trachea and bronchi of birds and mammals. In their immature stage the worms inhabit the lungs and air sacs. As the name suggests the males and females remain permanently joined in copula in the form of the letter 'y'. The most important and widely studied member of the genus is *S. trachea* [Montagu, 1811; Chapin, 1925], which occurs in the trachea and bronchi of many species of birds of different groups. The parasite is more or less cosmopolitan. In the Fauna of British India, Nematoda, Vol. 1, 1936, Baylis remarks, "The parasite is very widely distributed throughout the world, but the only record from the Indian region appears to be from the fowl at Colombo, Ceylon (v. Linstow)". In 1935 the author had opportunity to examine about a dozen chicken at Bareilly and recovered four specimens of gapeworms from the trachea of two of them. The worms on careful examination proved to be specimens of *Syngamus trachea*—a worm which has not been previously recorded from this country. One of the birds was found to be infested with *Catatropis indicus* Srivastava [1935]. In the mature state *S. trachea* lives in copula attached to the mucous membrane of the upper part of the respiratory tract. The eggs are laid by the gravid female while still in copula and pass out under the margin of the bursa of the male into the lumen of the trachea and bronchi. In their appearance the eggs differ from those of other strongyle worms in the presence of a thickened operculum at either end. They are in the 16-celled stage when laid. From the trachea they are coughed up into the mouth and swallowed and are eventually passed out with the droppings. Under suitable conditions of temperature and moisture the eggs usually hatch in seven to ten days. The larva undergoes a moult inside the egg shell. The second stage larva which retains the cuticle of the first stage is infective and one ecdysis is skipped over in the development of this parasite. It is, however, not necessary for the larva to

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hatch and infection may result by the ingestion of eggs containing fully developed larvae. Although the intercalation of an invertebrate intermediate host, such as earthworms, snails and slugs, in the life-cycle of this parasite is not necessary, they often act as reservoirs for the larvae. When larvae and mature eggs are ingested along with soil by earthworms they migrate from the gut to their muscular parts and remain alive there for over three years. The earthworm thus plays an important role in the collection of larvae from the soil, in carrying over the infection from season to season and in transferring them to the final host. On being swallowed by the final host, the larvae quickly migrate to the lungs where they undergo a further moult and sex differentiation and pairing take place. After pairing the worms leave the smaller air-passages and work their way to the large windpipes. Sexual maturity is attained in ten to fourteen days after the worms reach the trachea. The entire life-cycle is completed in a month or so.

The worms inhabit the trachea and bronchi of the turkey, fowl, pheasant, goose, duck, pigeon, guinea-fowl, pea-fowl, partridge and various species of other birds. Chicks, turkey poult and young pheasants are particularly susceptible and may die in large numbers. However, chickens invariably develop a very definite immunity when eight or nine weeks old, and once this age immunity develops any worms which may be present die and a reinfection is impossible. The turkey on the other hand does not develop any age immunity and may remain infested throughout. Turkeys do not show any symptoms, unless very heavily infested, and hence may serve as carriers for the parasite.

The 'gape' disease in young birds is probably mainly due to the mechanical blockage of the trachea, which is brought about by the presence of the parasite, local oedema of the mucosa at the seat of attachment and the accumulation of mucus. The migration of the larvae through the lungs does not appear to have any serious effect. Though the worms are blood suckers, the actual loss of blood is probably negligible. The symptoms are mainly those of suffocation. There is a peculiar cough or sneeze, the bird tosses its head, the neck is stretched forward with open beak in an attempt to take in more air. The death is usually due to suffocation though progressive emaciation is undoubtedly a contributory factor.

In the absence of any satisfactory treatment the practice of mechanically dislodging the worm, with or without the aid of certain chemicals, is widespread. A loop of horse hair is introduced into the trachea, twisted to entangle the worms and then withdrawn. The tip of a feather moistened with clove oil or turpentine is also often used in dislodging the parasite. In the case of valuable birds intratracheal injection of 1 c.c. Lugol's iodine or five per cent aqueous salicylic acid may be tried. Fumigation with such substances as heated carbolic acid, tobacco smoke or burning sulphur is of little value and may even be dangerous. Sodium salicylate at the rate of three drams to every quart of drinking water or pounded garlic in the proportion of one bulb per day added to the food of ten birds may result in the expulsion of the worms.

Prevention is much more important than treatment. Infested birds should be killed and the heads, respiratory and digestive organs burnt. Chickens acquire infection from turkeys, wild birds or from eggs and larvae carried over in the soil from previous infection. For about four to six weeks chicks should be raised on board floors and the droppings should be removed at regular intervals. They should never be kept on the same ground with turkeys. Moist localities where earthworms are likely to occur should be avoided. New chicks should be purchased out of clean flocks and quarantined. Infection from wild birds should be avoided. In this country snipes are suspected to serve as carriers.

The author is grateful to the Director and the Pathologist for much kind encouragement.

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# DISSEMINATION OF ANTHRAX INFECTION THROUGH DIRTY STAGNANT POOLS \*

BY

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(Received for publication on 28th May 1938)

(With two text-figures.)

ANTHRAX is a disease of great economic importance as it is enzootic in various parts of India and is responsible, during certain seasons, for heavy losses chiefly among horses, cattle, sheep and goats. It is also of importance in comparative medicine as it is transmissible to human beings through handling of infected materials.

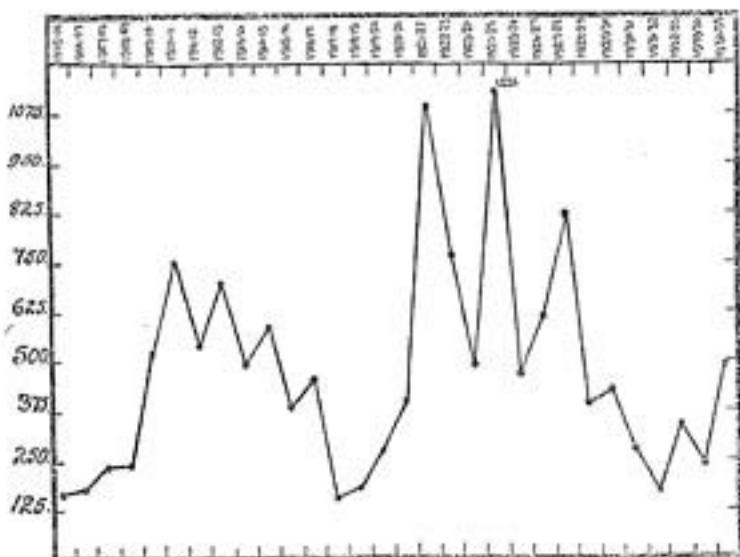


Fig. 1—Mortality by anthrax in cattle during thirty years ending 1934-35.

A study into the prevalence of this disease during the last thirty years in the Bombay Province reveals that it is enzootically prevalent in almost all districts of the Province and that there was a sharp rise and fall in the mortality

\* Paper read at the 24th Session of the Indian Science Congress, held at Hyderabad (Deccan), 1937.

curve (Fig. 1) during certain years, the cause of which is still unknown. It is prevalent throughout the year causing heavy mortality from April to September and in the month of December (Fig. 2). In this connection, it would be interesting to note that as an infection in the soil it is quite natural for Anthrax to break out in an epizootic form during the grazing season which, in most of the districts, begins from the latter half of June. The source of infection during the months of April and May, when practically no grazing is available, has, however, remained a mystery. It is not uncommon to find stall-fed draught animals also succumbing to Anthrax during the summer season and here again the source of infection was unknown.

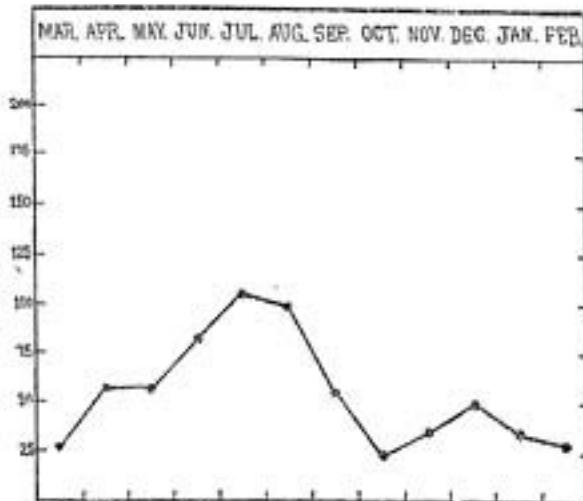


Fig. 2.—Anthrax in Cattle. Monthly average mortality. (11-years' average—1924-35).

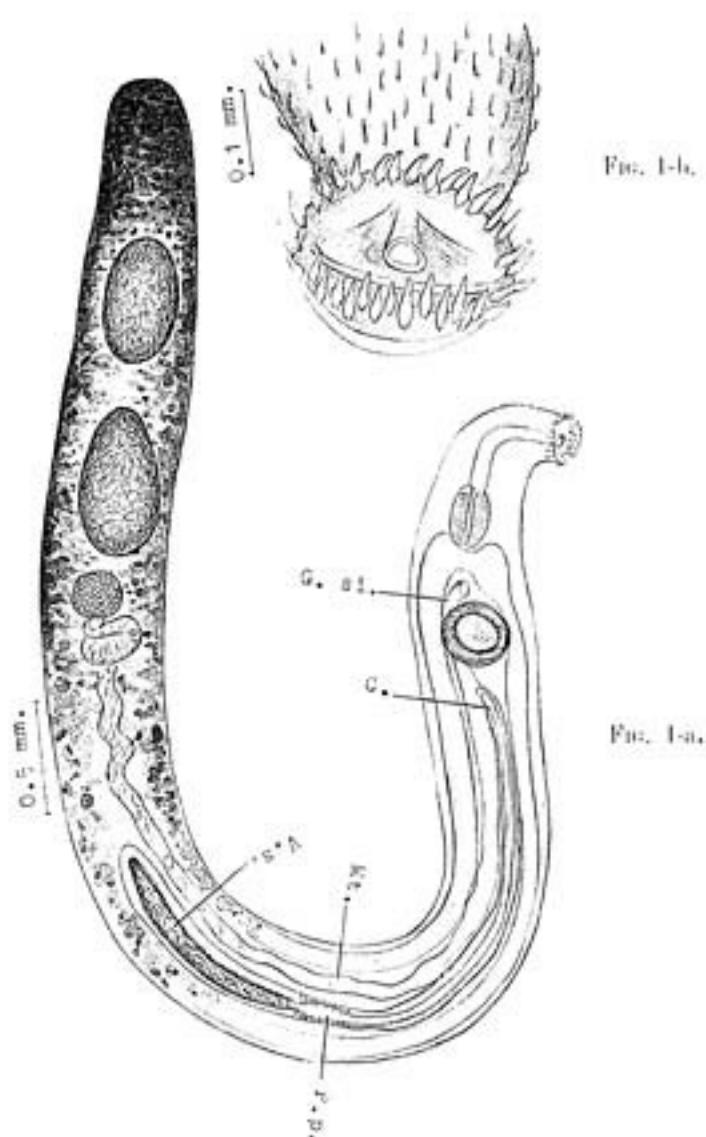
During the summer season of 1936 when green grazing was not available, a number of outbreaks of an undiagnosed disease was reported from the Ratnagiri district. Investigation which was carried out at the actual scene of these outbreaks revealed that the disease was Anthrax. The writer also detected a bullock which in spite of being daily yoked to cart and fed on dry foodstuffs had fallen a victim to this disease. A careful search for the source of infection was, thereupon, made with the results that stagnant pools formed in the beds of nullahs and rivulets appeared to be the only probable source as there was clear history that animals which had died of Anthrax were daily driven to such pools for watering purposes. An examination of these stagnant pools showed a thin layer of shiny brittle scum floating on the surface and dirty brownish water containing putrefying vegetable matter. Samples of water were collected from two pools, one at Lanja and another at Wilavade, and 5 c.c. of each sample were injected into a guinea-pig. The guinea-pig injected with the water sample of Lanja died within three days and its heart blood gave rise to a pure culture of *B. anthracis* on agar medium.

This, it would appear, is a very important finding in the epizootiology of Anthrax, inasmuch as water from stagnant pools has been proved to be the source of natural infection of the disease perhaps for the first time in India and that it will now be possible to check the occurrence of the disease especially during summer by adopting suitable preventive measures.

This work has been carried out under the auspices of the Imperial Council of Agricultural Research, New Delhi. The writer desires to express his gratefulness to Mr. E. S. Farbrother, M.R.C.V.S., I.V.S., Director of Veterinary Services, Bombay Province, Poona under whose guidance this work has been carried out. Thanks are also due to the Director, Imperial Veterinary Research Institute, Mukteswar for the confirmation of the strain of *B. anthracis* isolated by the writer from the stagnant pool.





FIG. 1-a. Ventral view of *Echimastephanus elouanae*.FIG. 1-b. Enlarged view of the head of *E. elouanae*.

- C..... Cirrus.
- G.si..... Genital sinus.
- Mt..... Metraterm.
- P.p..... Pars prostatica.
- V.S..... Vesicula seminalis.

A NEW PARASITE OF THE FAMILY ACANTHOCOELIDAE LUHE, 1909, FROM AN INDIAN HOST

BY

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(Received for publication on 25th March 1938).

(With Plate XVII).

Genus.—*Echinostephanus* Yamaguti, 1934.

*Echinostephanus cloacum*, n. sp.

Host—*Lates calcarifer*.

Habitat—Intestine.

Locality—Karachi and Puri.

It is a common parasite infesting the intestine of a food-fish found in the Arabian Sea and the Bay of Bengal. In the living state the parasite is light-brown in colour and possesses considerable power of contraction and expansion. The body is linear with a slightly attenuated neck and a rounded posterior end. It measures 6.12—8.92\* in length and 0.6—0.8 in maximum breadth which occurs usually at the level of the acetabulum. Usually the body has a nearly uniform breadth. The spines on the body are fairly large and have slightly hooked tips and triangular base. In the anterior region of the body of the parasite the spines measure 0.02—0.03 × 0.004 in size, but they become smaller in size and are fewer towards the hind end. Around the anterior end there are thirty-four cephalic spines, arranged in double alternating rows dorsally and in a single row ventrally and laterally. The spines in the dorsal rows are slightly larger than the rest and measure 0.057—0.061 × 0.015—0.019 in size. The cephalic spines on the ventral and lateral sides are of 0.045—0.05 × 0.015—0.019 size. The funnel shaped oval sucker 0.12—0.14 × 0.22—0.3 in size is anteriorly directed and bears the prominent oral spines described above. The oval sucker is followed by a prepharynx 0.5—0.6 long, and an oval pharynx 0.22—0.28 × 0.2—0.22 in size. The oesophagus is extremely short and in the contracted specimens it is hardly visible. The caeca are long and simple and open at the hind end into the lateral sides of the excretory bladder near its opening to the exterior. The acetabulum has a diameter measuring 0.34 and is situated at about the middle of the anterior third of the body length.

\*All measurements are in mm.

The two elliptical testes are situated in tandem and are separated from each other by a distance of 0·1—0·3. The anterior testis measures 0·52—0·8 × 0·26—0·44, and the posterior one 0·62—0·66 × 0·3—0·4 in size. The latter is situated at a distance from the posterior end not exceeding the length of the anterior testis. The cirrus sac is very long and tubular and may extend posteriorly up to half the distance between the ovary and the anterior end of the vitellaria. It encloses a tubular, 1·1—1·4 × 0·08—0·12, vesicula seminalis, a small pars prostatica, 0·2—0·3 × 0·04—0·06, surrounded by prostate glands, a long ductus ejaculatorius and cirrus. Terminally the cirrus opens close in front of the acetabulum. Almost the whole length of the ductus ejaculatorius may sometimes be found extended through the genital opening.

The ovary is small, 0·18—0·2 × 0·12—0·16, in size, and is situated close in front of the anterior testis. The receptaculum seminis is absent but the initial part of the uterus functions as the receptaculum seminis uterini. Laurer's canal is given off from the oviduct. The compact shell gland mass lies just in front of the ovary. The vitellaria are follicular and extend from the posterior end to a little in front of the posterior half of body. The follicles always meet in the median line behind the testes but in front of them they seldom meet mesially. The yolk reservoir lies dorsal to the ovary. The uterus is preovarian and contains a small number of eggs. The metraterm is a well-developed long, tubular structure almost the same length as the cirrus sac. The eggs are operculate, yellowish brown in colour and measure 0·06—0·073 × 0·038 in size.

The genus *Echinostephanus* was created by Yamaguti in 1934 with *E. hispidus* as the type species. The Indian form resembles the type species in most of its features but can be distinguished from it by the number and the arrangement of the oral spines. In the type species the oral spines are forty-two in number and are arranged in double alternate rows dorsally and ventrally and in a singular row laterally.

The author is grateful to the Director and the Pathologist of the Imperial Veterinary Research Institute, Mukteswar, for their kind encouragements.

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THE OCCURRENCE OF AN UNRECORDED FILARID  
NEMATODE, *ONCHOCERCA CERVICALIS* RAILLIET  
AND HENRY, 1910, IN THE LIGAMENTUM  
NUCHAE OF HORSES IN INDIA

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RAILLIET AND HENRY in a paper on the classification of the genus *Onchocerca* Diesing [1841], published in 1910 established a new species, *O. cervicalis*, which according to them was very common in France. Subsequently this nematode, which is commonly known as the neck threadworm, has been reported from equines in several countries,—Australia, England, United States of America and Africa. The parasite has been suspected to be the cause of fistulous withers and pole evil, having been found in a number of cases of the disease. The etiological relationship, however, between the worms and the disease has not yet been definitely established, as the parasite has also been met with in apparently healthy animals. Recently, Datta [1936] has demonstrated the invariable presence of an unsheathed microfilariae in the sections of affected skin from over twenty cases of Lichen tropicus in equines in this country. As in several other forms of Onchocerciasis, the microfilariae are not found in the blood of the diseased animals. Though the condition known as *khejlee* or Lichen tropicus in equines has been known in this country for a very long time, the occurrence of *O. cervicalis* in Indian hosts has not been previously reported. Through the kindness of Capt. Datta the author received some pieces of Ligamentum nuchae from cases of Lichen tropicus for examination. Entire ligaments from cases of the disease subjected to *post mortem* examination at this Institute were also examined. In three cases large numbers of inextricably coiled, fine, shining white worms were present. On a detailed examination all of them proved to be specimens of *O. cervicalis*. The adult worms usually occur between two lamellar portions of the Ligamentum nuchae. *In situ* the worms resemble the fibres so closely that it is almost impossible to follow the course of the parasite for any appreciable length during micro dissection. Small, hard, calcareous nodules varying in size from a pin head to a pea were often found associated with the parasite. Usually the anterior end was found embedded in the nodule.

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## 250 Occurrence of an Unrecorded Filarid Nematode in India

The life-history of *O. cervicalis* has been worked out by Steward [1933]. It has been demonstrated that certain biting flies, *Culicoides nebulosus*, commonly known as midges, serve as the intermediate host of the parasite. The midges take up the larval worms in the course of piercing the skin of infected horses and, after a period of twenty-four to twenty-five days, the infected midges contain larvae capable of infecting the final host.

No treatment for the destruction of the worms in the Ligamentum nuchae is known, and it is obviously difficult to prevent horses from being attacked by midges in localities where they occur. The avoidance of swampy pastures, however, may be beneficial in controlling the parasite.

The author is grateful to the Director and the Pathologist of this Institute for their kind encouragement.

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FIG. 1. *Micipsella indica*, n. sp. Worms in the clot of blood from portal vein (Actual size)

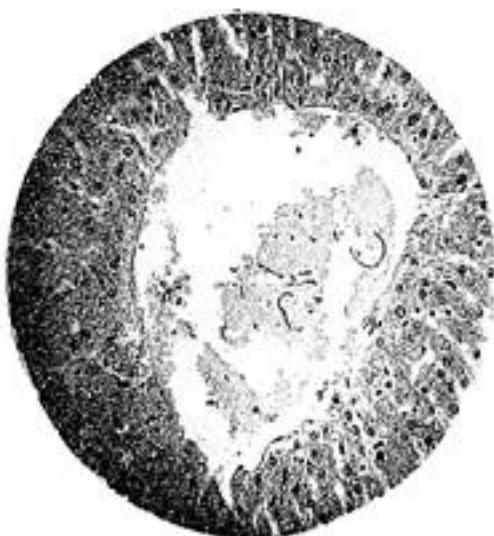


FIG. 2. Section of liver showing a dilated central vein containing microfilariae in blood  
 $\times 200$

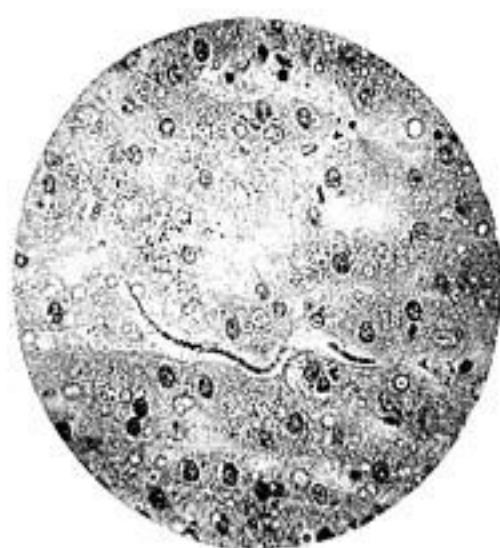


FIG. 3. Section of liver showing a microfilaria in a sinusoid  $\times 750$

*MICIPSELLA INDICA*, N. SP.

BY

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(With Plate XVIII and one text-figure)

SIX years ago, a piece of liver from a hare, *Lepus nigricollis*, containing a number of filariid worms was received from Tiruvannamalai, North Arcot district. The worms were in a clot of blood in the portal vein. Only some female worms were available for study then and because they resembled, to a very great extent, the females of *Micipsella numidica* [Seurat, 1917], they were recorded as such in the Annual Report of the Civil Veterinary Department, Madras, 1932-33. It has now been possible to obtain some male worms and a detailed study of these has shown some important differences between these and the ones described by Seurat.

Seurat [1917] described *Filaria numidica* which he obtained from the peritoneal cavity of *Lepus* species. In 1921, he created a new genus *Micipsella* to receive those worms and renamed them *Micipsella numidica*. Kalantarian [1924] described similar worms from rodents in Armenia and named them *Cercofilaria numidica* which, according to Yorke and Maplestone [1926], are synonymous with *M. numidica*. From the literature available, it would appear that so far only one species has been described.

A description of the male and female worms, obtained from the portal vein of the hare, is given below.

*Females*.—The length varies from 120 to 140 mm. The body is thread-like and gradually tapers at both ends, the head end being more blunt than the tail end. The cuticle is fairly thick and it is ornamented with two narrow rows of inconspicuous bosses running along the lateral lines from near the anterior end to almost the end of the worm. The mouth is a simple pore surrounded by a circle of very small papillary projections. A little behind the oral opening are four small submedium papillae. The oesophagus is uniform in its width and opens into a fairly wide intestine. The genital opening is a little in front of the level of the posterior end of the oesophagus. Opiosthodelphes. Uterus contains unsheathed larvae. Viviparous. The tail is slightly curved and its end is blunt.

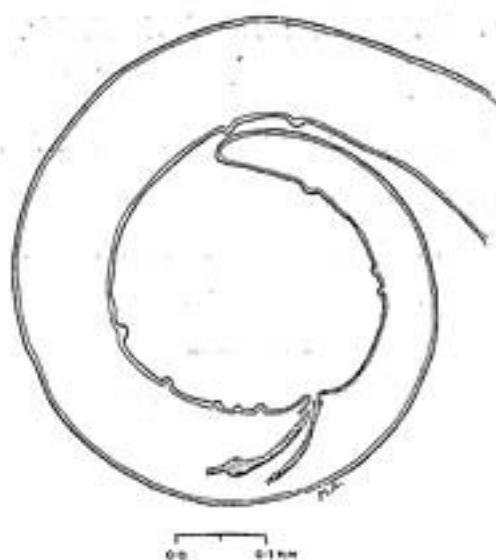


Fig. 1 *Micipsella indica* n. sp. Tail end of male worm.

*Male*.—70 to 100 mm. long. The mouth, oesophagus, and the ornamentation on the cuticle are as in the female. The tail end is spirally coiled and the end is bluntly pointed. There are six to seven pairs of pre-anal and three pairs of post-anal papillae. The spicules are short, subequal and dissimilar. The left one is 0.14 mm. and the right one 0.11 mm. in length. The left spicule has a distinct bulbous dilatation in its anterior third, the posterior two-thirds being tubular. The left one is tubular throughout but widens out somewhat at its distal extremity. The chitin of the posterior margin of each spicule is thicker than the opposite side.

Table I shows the differences between the two species.

TABLE I

| Name of worm                              | Cuticular ornamentation along lateral lines   | Spicules                                    | Post-anal papillae |
|---|---|---|--------------------|
| <i>Micipsella munidea</i> [Seurat, 1917]. | Two broad rows of conspicuous bosses. The tail of male is covered on both dorsal and ventral surfaces with minute papillae in addition to above mentioned rugosities. | Sub-equal, similar. Both have pointed ends. | Two pairs.         |

TABLE I—*concl.*

| Name of worm                      | Cuticular ornamentation along lateral lines  | Spicules   | Post-anal papillae |
|-----------------------------------|--|--|--------------------|
| <i>Micipsella indica</i> , n. sp. | Two narrow rows of inconspicuous bosses. The cuticle of the tail of male has no papillae on dorsal or ventral surfaces excepting the rugosities mentioned above. | Sub-equal, dissimilar. The left one is longer and has a distinct bulbous enlargement in the anterior third. End pointed. Right one tubular throughout, but the end widens out to some extent to make it appear blunt at its extremity posteriorly. | Three pairs.       |

The above table shows clearly the differences between the two parasites, so it is proposed to make a new species of the worm described and to name it, *Micipsella indica*.

*Specific diagnosis.*—Female 120 to 140 mm. long. The simple mouth is surrounded by a circlet of minute papillary projections. Cuticle ornamented with two narrow rows of papillae following a more or less zigzag course along the lateral lines. Vulva, a little in front of the posterior end of the oesophagus. Opisthodelphes. Unsheathed larvae in uterus. Viviparous. Male 70 to 100 mm. long. Mouth oesophagus and cuticular ornamentation as in female. Tail spirally coiled. Six to seven pre-anal and three post-anal papillae. Spicules subequal. The left one is longer than the right and has a distinct bulbous enlargement in its anterior third. The right one tubular with its posterior end broadened somewhat.

*Host.*—*Lepus nigricollis*.

*Location.*—Portal vein.

*Locality.*—Tiruvannamalai, North Arcot district, Madras Presidency.

The liver showed passive congestion. It is not known if microfilariae are met with in the peripheral blood of infected hares.

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## THE OCCURRENCE OF *PARAGONIMUS WESTERMANNI* IN THE LUNGS OF CATS IN INDIA

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(Received for publication on 25th May 1938.)

THE lung fluke of mammals—*Paragonimus westermani*—has long been recognised as an important parasite of man and animals both, domestic and feral. It is of considerable veterinary importance, and its importance from public health point of view is even greater. The parasite was first discovered during autopsy in the lungs of a Bengal tiger which had died in the Zoological Gardens, Amsterdam, Holland, in September, 1877. The specimens were sent by the Director, Westermann, to Kerbert, who named them *Dist. westermani*. Three years later Kerbert received, through Bolau, specimens of the same parasite recovered from the lungs of a Bengal tiger in the Zoological Gardens at Hamburg, Germany. In 1879, Ringer found a parasite in the lungs of a Portuguese resident of Formosa who had died of rupture of aortic aneurysm. The material was sent to Manson who recognised it as a distomate fluke. A year later Manson found large operculate ova in the rusty, blood-flecked sputum of a Chinese suffering from haemoptysis, who had lived in Northern Formosa. A few years later Yamagiwa and other Japanese workers found mature flukes in atypical foci in the body, including the brain, where their presence was accompanied by symptoms of Jacksonian epilepsy.

Appreciating the inadvisability of retaining this parasite in the old group *Distoma*, Braun [1899] created the genus *Paragonimus* for it, which is assigned to the family *Troglotrematidae* Odhner [1914]. In recent years the parasite has received considerable attention at the hands of several workers. The morphology, life-history, pathogenicity and the geographical distribution have all been studied in detail. The definitive hosts of the parasite other than man are pig, dog, cat, goat, cattle, tiger, panther, mink and numerous other wild carnivores. A number of papers have been published on the distribution of lung flukes. Recently, La Rue and Ameel [1937] have summarised our present knowledge of the geographical distribution of the parasite. Although this fluke has not so far been reported from man in this country, it must be looked upon as a potential human parasite, which awaits only a change in the diet of human beings to assume considerable importance. Surveyor [1919] recorded an apparently

imported case of paragonimiasis in a Chinaman in Bombay. The only record of the occurrence of this parasite in domestic animals in this country is by Rao [1935] who found two dogs in the Madras Presidency harbouring the parasite in their lungs. During the routine *post mortem* examination, the author recovered a pair of flukes from a cyst in the lungs of a cat, which on examination proved to be specimens of *P. westermani*. This is the first record of the occurrence of lung flukes in Indian cats.

Usually the parasite occurs encysted in pairs, specially in animals. The eggs are laid in the cysts and escape through the connecting channels into the bronchi, or else when the cysts rupture. They are either voided with the sputum or are swallowed with it and passed out with the faeces. Under suitable conditions of temperature and moisture they hatch in four to seven weeks. The actively swimming miracidia on coming in contact with a suitable molluscan host penetrate into its soft tissues. Inside the body of the snail they undergo further development into sporocyst, rediae and cercariae. The cercariae are microcercous, with an elliptical body and short, knob-like caudal appendage. On emerging from the snail these larvae swim about and if a crayfish or a suitable crab be available, penetrate into their soft parts and encyst. The final host acquires infection usually through eating the second intermediate host harbouring metacercariae. Another possible, though not common, mode of acquiring infection is by swallowing free, viable cysts in drinking water. The cysts remain viable in running water for at least twenty-five days. It is quite easy to surmise that, under natural conditions, cysts could be freed from decomposing crayfishes in situations accessible to the final hosts. The adolescaria emerges from the cyst in the duodenum of the final host. It penetrates through the wall of the intestine, traverses the abdominal cavity and migrates upwards through the diaphragm into the thoracic cavity, where it penetrates through the pleura into the lungs and finally arrives in the bronchioles. In the lungs it becomes encysted and attains sexual maturity.

Though the normal habitat of the parasite is the lungs, it is not infrequently generalised in its distribution in the whole body, being found in the liver, intestinal wall, mesenteric glands, muscles, testes, brain, or attached to peritoneum or pleura. It is in these atypical foci that the parasite is most harmful and difficult to detect. Parasitic cysts in the latter situations work their way to mucous or epithelial surfaces, such as the intestinal mucosa, biliary tract epithelium, pleural or peritoneal surfaces, or even skin, in which positions they may form ulcers which heal with difficulty. In one case the author found eggs of this parasite embedded in the cardiac muscles of an Indian dog. No satisfactory treatment is known. Tartar emetic may relieve pulmonary symptoms in human beings, but the parasites in the cysts are not affected.

The author is grateful to the Director and the Pathologist for their kind encouragement.

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## ABSTRACTS

**Time of cutting hay, and losses during hay-making.** WATSON, S. J., FERGUSON, W. S. AND HORTON, E. A. (*J. Agri. Sci.* 27, 224, 1937.)

The yield of nutrients from hay cut at two different stages of growth, early and ordinary, and losses occurring during the making and stacking were determined. The ordinary hay was cut when the majority of the grasses had flowered and the earlier grasses had shed their seed. The early type was cut some three to five weeks earlier when the majority of the grasses were just coming into flower. The growth which took place on the early hay plots between the times of cutting the early and ordinary hay was also considered in estimating the early hay-yields.

The experiments were carried out for five years and sheep were used to determine the digestibility of the fresh grass and hay samples.

The average yield data for the five years (1931-35) show that the average production of ordinary hay was 992 lb. more than the early hay including the aftermath. The production of starch equivalent was greater in the ordinary hay by 249 lb. per acre, but the early hay yielded 47 lb. per acre more protein equivalent.

The ordinary hay conserved 522 lb. more starch equivalent and only 11 lb. less protein equivalent than the early hay if the aftermath is excluded. The authors think that the early hay cannot compete with the ordinary hay when the yields of nutrients in the hays alone are considered. The average total losses of dry matter, starch equivalent and protein equivalent in the early hay were 23·2, 41·3 and 32·0 per cent and in the ordinary hay 20·0, 32·0 and 29·4 per cent respectively. The poorer weather conditions at the time of making the early hay probably account for the slightly greater losses suffered by the early hay. Adverse weather conditions caused high losses, the highest losses of D. M., S. E., P. E. being 36·7, 58·7 and 53·7 per cent respectively. The losses during the curing process in the stack were of minor importance compared to the losses in the field. In some cases, the digestible nutrients appear to have increased as a result of the fermentation in the stack.

The methods of windrow and tramped heap or pike drying for making "seeds" hay were also compared. Pike hay was slightly more digestible and gave a greater yield of S. E. and P. E. [N. C. D. G.]

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**Ovine encephalitis associated with *Listerella* infection.** ERWIN JUNGHERR, (*J. A. V. M. Ass.* xci, 73-87.)

This author describes a small epizootic of meningo-encephalitis among well-nourished mature lambs in a flock of sheep associated with a *Listerella*-like organism, similar to those reported by Seastone in U. S. A. and by Gill in "Circling Disease" in New Zealand. The outbreak involved four sheep within a period of about twenty days. This was preceded by an isolated case about one year earlier.

A combination of the following symptoms characterised the clinical disease:—fever, anorexia, restlessness, lameness, lateral deflection of the head, staggering, prostration, excitability, coma and death.

Centrally located polynuclear foci, monocytic perivascular and meningeal infiltration in the medulla oblongata and slight parenchymatous and fatty changes in the liver were the chief histological changes noticed. Groups of *Listeria-like* organisms were seen in the polynuclear foci of the medulla of two cases.

The organism to which the author ascribes a causal significance was cultured from the medulla of two cases. It is a Gram-positive non-spore-forming rod with rounded ends, measuring  $1.7 \times 0.7 \mu$  in average. It is sluggishly motile. On liver agar slants, colonies measure 1 to 3 mm. in diameter, are smooth and low-convex, have entire margins and are of a milky bluish colour. It is negative for the indol, hydrogen sulphide, Voges-Proskauer and nitrate reduction tests. It is methyl-red positive. Litmus-milk is reduced but gelatine broth is not liquefied. In a base of extract broth, acid, but no gas, is formed from the following sugars:—glucose, inositol, maltose, mannose, and salicin. Delayed fermentation occurred in arabinose, galactose, glycerol, lactose, mannitol, raffinose, rhamnose, sorbitol, sucrose, trehalose and xylose. Dulcitol and inulin are not attacked.

The strains isolated from this outbreak, excepting certain minor differences in fermentation reactions, were found to be indistinguishable from the Princeton and New Haven strains of *Listeria* by cultural, agglutination and agglutination-absorption tests.

Intranasal injections of brain suspensions and of small doses of culture in mice reproduced, after an incubation period of about two weeks, disturbances similar to those observed in sheep. Larger doses of culture produced meningitis and rapidly fatal septicaemia. Mice inoculated intravenously died within twenty-four hours. Suspension of culture which had been maintained on laboratory media was pathogenic to a rabbit injected intranasally. Sheep infected by intranasal and conjunctival application of culture showed a marked thermal and agglutinative response, but recovered: a similar but milder reaction was observed in a control animal kept in the same pen. Intracurotid injection in sheep caused death from haemorrhagic meningitis within forty-eight hours. [V. R. R.]

**Dicalcium phosphate and steamed bone flour as supplements for a phosphorus deficient ration.** W. GODDEN RAY and S. C. RAY. (*The Emp. J. of Exptl. Agri.*, Vol. VI, No. 21, 1938, pp. 79-84.)

EXPERIMENTS were carried out with sheep with the object of determining the actual utilisation of phosphorus in the forms of dicalcium phosphate and steamed bone flour when used as phosphatic supplements to a basal ration markedly deficient in phosphorus. Four Oxford cross wethers were used for the experiment and the ration consisted of chopped oat straw, dried beet pulp, starch and blood meal which had a  $P_2O_5$  content of about 0.14 per cent. The experimental work was divided into four periods when (a) Basal ration, (b) Basal ration plus dicalcium phosphate, (c) Basal ration and (d) Basal ration plus steamed bone flour was provided to the animals.

Blood samples were analysed at regular intervals and the data were indicative of the periods when animals were showing marked signs of phosphorus deficiency or, alternatively, had responded to the use of phosphatic supplements.

In the initial period there was a marked loss in condition and impairment of appetite but progressive improvement ensued as soon as dicalcium phosphate was introduced in the ration. When, however, dicalcium phosphate was eliminated from the ration, the blood picture indicated a marked phosphorus deficiency and also low values for inorganic P in blood and serum and raised values for calcium and phosphates in the serum. No inappetence was, however, noticed in the experimental animals. The introduction of bone flour at this stage again exerted a corrective influence and the blood picture rapidly returned to normal.

The experimental evidence leads to the conclusion that phosphorus supplied in the form of dicalcium phosphate is utilised to an extent approximately 50 per cent greater than when supplied in the form of steamed bone flour, and that dicalcium phosphate added as a supplement to a phosphorus deficient ration appears to exert a more favourable influence on nitrogen assimilation than does steamed bone flour.

The higher actual retention of CaO noticed when steamed bone flour is used as a supplement is due to more CaO supplied by this supplement as compared with dicalcium phosphate. In regard to the percentage retention of the CaO ingested, there is hardly any difference between these two supplements. [H.B.S.]

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**The Cattle of the Gold Coast.** J. L. Stewart. (*The Emp. J. of Exptl. Agri.* Vol. VI, No. 21, 1938, pp. 85-94).

In this interesting article the author gives a comprehensive description of the various types of cattle in the Gold Coast and refers to the system of animal husbandry, the contemplated plans of improvement and associated limiting factors.

The present cattle population of the Gold Coast is estimated at nearly 200,000 and of these 160,000 are in the northern territories, nearly 40,000 in the plains of the eastern parts of the Colony and a few scattered herds in other regions. The best cattle are found in the north-east of the northern territories.

The distribution of cattle in the different regions of West Africa is correlated with the degree of trypanosomiasis infection and availability of water. Shortage of water has seriously inhibited the development of the cattle industry, but efforts are now being made to remedy this short-coming in all the livestock areas.

The origin of the unhumped cattle of West Africa has been traced from three types, the Hamitic Longhorn, the oldest African bovine, the Brachyceros or dwarf shorthorn imported from Asia many centuries ago and the Zebu more recently brought from Asia. The Zebu features are conspicuous in animals in northern territories, whilst in the remote bush areas along the coast shorthorn blood is predominant. The present grading up policy has a particular prejudice in favour of the Hamitic Longhorn type due to its greater resistance to trypanosomiasis and other enzootic diseases as compared with the Zebu. Another excellent type which is highly favoured in the tsetse areas and is nearest to the pure Hamitic Longhorn is the N'Dama breed. It appears to be the most suitable bovine for improvement work in the tsetse areas, as it is the only type in West Africa which can usually stand to the infection of different strains of trypanosomes. N'Dama is probably an admixture of the Zebu and Brachyceros.

Two types of Zebu are met with in West Africa. There is the white Fulani, a Nigerian type (weighing 8½ to 10 cwt.) originating probably from the fusion of the Zebu proper and the Hamitic Longhorn and the other is the Sudanese or shorthorned Zebu, a result of the fusion of the Zebu and *Brachyceros* strains.

Another type known as Sanga is a cross between humped Zebu and unhumped West African Shorthorn—a name coined by the writer for the cattle of the Gold Coast which are not unlike miniature British shorthorn cattle. If Sanga are inter-bred, there is no reversion to the original parent stock. These animals are suitable for draught as well as for meat. The Zebu and Sanga cattle are reared in areas where *Tsetse* infestation is light.

The primary aim of cattle improvement in West Africa is to raise the weight of West African Shorthorn bullocks from 5 to 7 cwt. and this has proved to be quite easy under the existing conditions of husbandry by means of the use of improved bulls. Early attempts at improvement by the introduction of exotic sires as, for example, Hereford, Shorthorn, Kerry, etc. ended in failure and the plans of improvement are now directed towards grading up the indigenous livestock. In this policy the Hamitic Longhorn types, principally the N'Dama, are playing an important role. Breeding work is carried out at Pong-Tamale, the headquarters of the Department of Animal Health, and also at Native Administration Farms, which are self-controlled but advised by the Department. There are now eight successful Native Administration Farms and over twenty more are in process of establishment. Improved bulls are issued by the Native Administration Farms as well as by the main Government Farm.

Tending of communal herds is carried out satisfactorily by hired Fulani herdsmen who have taken to cattle rearing as a profession for generations. These men are conversant with the basic facts of animal husbandry, realise the importance of a good sire and are familiar with good pastures. Unfortunately their number is still small and herd management in a great number of cases is in the hands of ignorant small boys.

Epizootics and entozoon infections have acted as the most serious limiting factors to any contemplated plan of improvement. Between 1930 and 1934 effective campaigns of prophylactic vaccinations were launched against rinderpest and considerable progress has since been made in regard to the control of this serious cattle menace. It is a routine practice now to immunize two year old cattle at district camps. Against contagious pleuro-pneumonia too an effective vaccine is now available. Trypanosomiasis, however, is still a serious menace and a difficult hurdle to negotiate in any plan of cattle improvement in West Africa. [H. B. S.]

## NOTES

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### The Maynard-Gangaram Prize

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APPLICATIONS are invited for the "Maynard-Gangaram Prize" of the value of Rs. 3,000 which will be awarded for a discovery, or an invention, or a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality and Government servants are also eligible for it. Essays and theses are not eligible for competition and applicants should prove that some part of their discovery, invention etc., is the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All entries in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1938.

### Prize for Improved Agricultural Implements

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In order to encourage inventors to improve existing implements of cultivation and to design new implements and machines better suited to Punjab conditions and within the power of the average cultivator to purchase, the Punjab Government have decided to institute a scheme of prizes. Each year during the scheme, applications will be invited for a suitable design of particular improved agricultural implement or machine. These prizes will be open to all, irrespective of nationality. Government servants can compete, subject to the consent of the Government under whom they are employed.

During the current year a prize of Rs. 3,000 is offered for a suitable design for a bullock-drawn cultivator. The implement must be simple in design; cheap in cost, so as to be within the purchasing power of an average cultivator; capable of repairs by an average village blacksmith; efficient in stirring up fallow land quickly after rain or irrigation, in order to conserve the maximum amount of moisture in the soil, and suitable for the inter-cultivation of crops sown in lines.

The cost of manufacturing the cultivator must not exceed Rs. 15.

Competitors must submit applications setting forth the advantages claimed for their respective designs and accompanied by scale drawings and specifications which must be sufficiently complete in all details to enable a manufacturer to make the implement.

The applications will be examined by an Expert Committee which will select for manufacture for trial purposes designs which hold promise of sufficient merit. Applicants whose designs are so selected will be required to deliver the implement in complete working order at Lyallpur or elsewhere in the Punjab within one month of receipt of instructions. Actual pocket expenses up to a maximum of Rs. 50 will be allowed. The award of the Committee will be final.

The entry, for which the prize is awarded, will become the sole property of the Punjab Government which also reserves the right to postpone or withhold the award of the prize, if no entry of sufficient merit is received.

Applications, complete in all respects, must reach the Director of Agriculture, Punjab, Lahore, by 31st October, 1938, at latest.

# PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

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## 1. Agriculture and Live-stock in India

- ICAR. 1 A bi-monthly journal of agriculture and animal husbandry for the general reader interested in agriculture or live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9s. 9d. per annum. Price per part Rs. 2 or 3s. 6d.). Volumes I to VII complete are available.

## 2. The Indian Journal of Agricultural Science

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- ICAR. 10-7 No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng and the Isolation of Types Resistant to the Disease, By W. McRee, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4s. 3d.
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- ICAR. 10-12 No. 12. The Fungi of India, Supplement I. By B. B. Mundkur. (In the Press.)

#### 5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

- ICAR. 8-1 No. 1. List of Publications on Indian Entomology, 1930. Compiled by the Imperial Entomologist, Pusa. (1934). Price As. 14 or 1s. 6d.
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- ICAR. 8-18 No. 18. Milk Records of Cattle in Approved Dairy Farms in India. By K. P. R. Kartha. (*In the Press.*)
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#### 9. Miscellaneous Publications

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ARL7.187 The Production of Cigarette Tobacco by Flue-curing. By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Res. Publ. Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1s. 9d.

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#### 10. Catalogue of Indian Insects

The following Parts have been issued:—

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ICAR. 11-3. 3. Bombyliidae, by R. Senior-White. 1923. Price, As, 8.

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 ICAR. 11-23. 23. Chalcidoidea by M. S. Mani, M.A. (Res.), 1937. Price, Rs. 3-2 or 5s. 6d.

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Purchasers not residing in Asia, should apply to the High Commissioner for India, India House, Aldwych, London.

**NOTE.—**When indenting please give only the symbol preceding the name of the publication.



## ORIGINAL ARTICLES

### "ACTINOMYCOSIS" \* AND ACTINOBACILLOSIS IN ANIMALS IN INDIA †

BY

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(Received for publication on 4th May, 1938)

(With Plates XIX—XXIV.)

THE object of writing this paper is to call the attention of veterinarians in India to the existence of "Actinomycotic" disease-conditions in animals, and to emphasize the necessity of making a scientific diagnosis and allotting the correct nomenclature to these conditions. For this purpose, a résumé of the literature is also placed before them.

It appears that, in this country, the clinical side of the subject has been sadly neglected, the few cases so far recorded indicating that the disease, which is referred to broadly as "Actinomycosis", was in most cases, discovered on microscopic examination of material collected *post mortem*. A previous clinical diagnosis of the disease has been suggested only in a few cases, while in others, a chance finding of the actino-body has been instrumental in the discovery of the "actinomycotic" character of the granuloma. It may be argued that the disease is of rare occurrence in India, but the records of this Institute show that, contrary to the belief of veterinary research workers in the past, typical cases of 'Lumpy Jaw' and 'Wooden Tongue' were not uncommon in North Bihar. Similarly, enquiries made, by the author, of students who visit this Institute for training have elicited the information that conditions resembling "Actinomycosis" in cattle do occur. It was suspected, however, that the clinical diagnosis was usually not a scientific deduction and that attempts to have such diagnosis confirmed microscopically were seldom made. Under such circumstances, one need not wonder at the paucity of material for cultural examination.

In this Institute, only fixed tissues have been received which have shown, often unexpectedly, the presence of an "actinomycotic" disease. It will be realised that in interpreting such granulomata, one has to go by the results of work done in other countries without an opportunity to take up independent research. It is highly probable that it is not the rarity of "actinomycosis"

\*Although not in favour of the use of the non-specific comprehensive term "Actinomycosis", the author has used it in this article for the sake of brevity and has indicated such use by placing the term usually within inverted commas, as above.

†Presented at the 25th Session of the Indian Science Congress, Calcutta, 1938.

but the prevalence of economically more important diseases and the lack of facilities available to the average veterinarian in India which are standing in the way of such research.

The Annual Report of the Civil Veterinary Department, Madras Presidency, 1927-28, records the finding of typical "Actinomycosis" in a three months old lesion "in the jaw" of a bullock and the finding of an acid-alcohol-fast *Streptothrix* in sections of lime-sized growths "in the left shoulder blade" of a buffalo. Datta [1933] has recorded the finding of "Actinomycosis" in a buffalo-heifer in growths "on the parotid glands of both sides". The specimen referred to by him has been studied further by the author and its description will appear later (p. 278).

The *Streptothrix* has been seen in association with lesions of other diseases of Indian animals and has even been highly suspected as the primary etiological factor [Datta, 1931; Ayyar, 1925]. But, as Datta [1933] has clearly stated, "reports on genuine cases of actinomycosis of animals have been very few indeed". Apparently he has been able to discover only two such previous reports viz., that of Pease [1891] who described an outbreak amongst buffaloes in the Punjab, and the one mentioned above from the Civil Veterinary Department, Madras (1927-28).

During routine examination of specimens sent to the Pathology Section of this Institute, the author had the good fortune to encounter a few specimens of "Actinomycosis" and became interested in their etiological classification. This necessitated a re-examination of the entire, relevant, catalogued material spread over the last fifteen years. It was realised that in the absence of complete clinical histories of the cases, and also of any cultural material, such a classification was not without risks of contradiction. Nevertheless, it was not considered altogether impossible in all cases.

Workers in other countries have explored several etiological factors which may produce structures that are commonly known as "actino-bodies" or "ray-colonies". The essential feature of these is a peripheral zone of radiating clubs. It must be borne in mind, however, that this essential part of the ray-colony is not necessarily a portion of the etiological organism. Experience [Bostroem, 1890; Brumpt, 1906; M'Fadyean, 1889; Delepine, 1890] has shown that the formation of clubs as a radiating peripheral zone may be brought about as a result of defensive, or according to some [Crookshank, 1888; Gibson, 1934; Lignieres and Spitz, 1902] offensive mechanism of the organism against the tissue, or as a result of tissue-reaction against the invading parasite. "Christiansen, and others before him, have shown that actinomycotic formations can be produced by moulds. Other writers have shown them present with tubercle bacilli. Magrou [1919] produced" such formations by inoculating Staphylococci into testicles of guinea-pigs..... "Thus, the mere presence of club-bearing granules is no proof that there is a question of Actinomycosis" [Magnusson, 1928]. Actino-bodies may be met with in several unrelated disease conditions : they occur, for instance, in true actinomycosis, in actinobacillosis, in chronic staphylococcal infection of the

skin or udder [Albiston and Pullar, 1934], around Streptococci [Albiston and Pullar, 1934], possibly in some Corynebacterial granulomata, around dead tubercle bacilli [Meyer and Mayer, 1927], around worm eggs (*Schistosoma*) and following intravenous injection of tellurium in oily suspensions [Levaditi and Dimanesco-Nicolau, 1926]. It will be apparent then that for the etiological agent one has to search under the peripheral zone of the actino-body. The causative organism is situated centrally in the young lesion but in older colonies the most central portion may have undergone degenerative changes, whereas the middle zone shows what can be interpreted as an active vegetative area of the etiological factor. In some cases the micro-organism may be seen situated just under the peripheral zone or even scattered amongst the bases of the clubs.

When one takes these facts into consideration there seems little doubt that with the knowledge of the morphology and staining peculiarities of micro-organisms one could determine the etiology of the actino-body with some degree of certainty by comparison with other known granulomata with similar microscopic appearance. Thus the ray-colonies could be etiologically classified without the need, in every case, of a cultural examination. Indeed, speaking of true actinomycosis, Colebrook [1931] asserts that a diagnosis of actinomycosis could thus be given, for in practice "such cultivation is not absolutely necessary because just as in tuberculosis the demonstration of acid-fast bacilli is sufficient for a diagnosis which will be correct ninety-nine times out of a hundred, so in actinomycosis the finding of definite granules visible to the naked eye and composed of Gram-staining mycelium will make it legitimate to infer the presence of an infection by *Actinomyces bovis*". There is little doubt that a similar view could be taken of other "actinomycotic" conditions, especially, if we consider the works of Griffith [1916], Bosworth [1923] and Magnusson [1928].

This brings us on to the subject of nomenclature to be adopted. The word "actinomycosis" has been in use for a long time and is so closely connected with research in this direction that by constant but loose usage, it continues to designate even to-day the entire group of diseases, characterised by the formation of granules, wherein actino-bodies are found. Various authors [Colebrook, Bosworth, Davies, Magnusson, M'Fadyean] have from time to time suggested revision of terminology but their suggestions have not been universally adopted, partly on account of the fact that the comprehensive use of the term "actinomycosis" has become firmly established and partly because some of the advocates of revised taxonomy have allowed themselves to adopt the old terminology or have suggested terms which are defective in expression.

It is well known that until the discovery of the *Actinobacillus* by Ligierres and Spitz in 1902, the etiological difference between actinomycosis and actinobacillosis was not realised. Bollinger [1877], who was the first to give a scientific account of "actinomycosis", had before him the diseased jaw-bone as well as tongue and affected lymph glands of the head. Until 1902,

scientific workers believed that all that showed the actino-body was actinomycosis due to the Gram-positive *Streptothrix*, and that the same disease attacked the jaw-bone and tongue. In fact, Bollinger had "no doubt that in the mycosis of the jaws, the corresponding lymph glands are similarly affected" [M'Fadyean, 1932]. The description and figures of "actinomycosis" given by nineteenth century authors were correct in so far as the presence of the actino-body was concerned, but they gave little help as to whether the condition depicted was true actinomycosis or actinobacillosis [M'Fadyean, 1932].

Although Wolff and Israel had already cultivated the anaerobic organism of true bovine actinomycosis in 1891, the importance of the discovery of the *Actinobacillus* was not promptly realised. Wright [1905] in his memoir cast a doubt if there was such a thing as a Gram-negative bacillus capable of causing an "actinomycotic" disease in bovine. Although Nocard [1902] had verified the discovery of Lignieres and Spitz, Wright was anxious to have it confirmed. Further, Schlegel [1928] in Kolle, Kraus and Uhlenhuth's "Handbuch der Pathogenen mikroorganismen", suggests that the appearance of the actinobacillus results from the method of staining only. With improvements in laboratory methods, however, the two etiologically distinct diseases due to the Gram-positive fungus (actinomycosis) and the Gram-negative bacillus (actinobacillosis) are recognised to-day.

It may be mentioned here in passing that *Actinomyces bovis*, the anaerobic species of Wolff and Israel is the cause of true actinomycosis in animals and in man, and that the aerobic type of actinomyces cultivated earlier in 1890 by Bostroem, and commonly known as *A. bostroemi* (*A. hominis* or *A. graminis*) is recognised to-day by most scientific workers as a mere saprophyte of the soil, having no etiological relationship with true actinomycosis. In spite of the fact that the aerobic organism is known to grow readily on ordinary culture media, Bostroem [1890] could obtain it only in twelve out of 700 tubes inoculated with material from eleven cases of bovine actinomycosis. Further, the organism has failed to infect laboratory animals and cattle. The species of Bostroem should, therefore, be looked upon as an air contaminant and one may "seriously question the relationship of the aerobic form to the disease actinomycosis" in man and animals [Henrici, 1930]. Moreover, Colebrook [1921] states that *A. bovis* is a rather delicate (easily killed by drying), non-sporulating anaerobe which has never been met with outside the host-body, thus being an obligatory parasite. In support of Magnusson's [1928] statement regarding actinomycosis in man and animals, Davies [1932] mentions that *A. bostroemi* has never been isolated from bovine lesions and adds that "It has never yet been shown that *A. bovis* can lead a saprophytic existence". As early as the end of the nineteenth century, Crookshank [1888] and Delepine [1890] had suggested the marked similarity between the etiological agents of true human and bovine actinomycosis. The same view was emphasized by Wright [1905] and is generally accepted to-day, the organism isolated from different parts of the world being *A. bovis* [Colebrook, 1931]. The difference noted by older

authors between the Gram-staining characters of the clubs in human and bovine lesions [Wooldridge, 1907] is no longer recognised. The occurrence of pus formation in the human and its rarity in the bovine lesion is explained by M'Fadyean [1889] on grounds of a host-specific tissue reaction, on analogy with tuberculosis. The failure of Iodine therapy in human actinomycosis [Colebrook, 1921] is easily explained [M'Fadyean, 1932], because the treatment is a success not against actinomycotic infection but against actinobacillosis in animals. Although the bone does not respond to internal medicinal treatment in most infections, success with iodine therapy in some bone lesions of animals may be due to the fact that the infective agent was the bacillus and not the streptothrix. According to M'Fadyean [1932] *Actinobacillus* is not known to be pathogenic for man and Griffith [1931] states that "there is no authentic record of a human case"; but Colebrook [1930] cites one case of meningitis stating that it is not impossible that other cases have been overlooked. Also, Beaver and Thompson [1930] refer to two previous cases and describe, in addition, one case of fatal infection.

Resuming the subject of nomenclature, a study of the works of Magnusson [1928], Albiston [1930], Albiston and Pullar [1934], and Davies [1935] makes it appear beyond any shadow of doubt that there occurs in the bovine and porcine udders a chronic Staphylococcal granuloma with typical actino-bodies possessing a peripheral zone of radiating clubs. Statistical data tend to show that in the bovine host infection is always of the Staphylococcal type. Albiston and Pullar [1934] state that of 153 cases of "actinomycotic" mastitis of bovines described from different parts of the world, 151 were due to *Staphylococcus*, one due to *Actinobacillus*, and one due to an unidentified *Streptothrix*. In the last case "the skin over the udder showed actinomycotic lesion, and the mammary infection was obviously an extension from the skin lesion". On a number of occasions a pure culture of the staphylococcus (*S. pyogenes*) has been obtained by various workers from lesions of so-called bovine "actinomycotic" mastitis. In one case the culture so obtained has been successfully used by Hulphers [1923] in producing the original disease in a bovine. The success which attended Magrou's experiments [1919] has proved that the Staphylococcus is capable of producing a chronic granuloma closely resembling "actinomycosis". This then would lead one to recognise a chronic staphylococcal "actinomycosis" of the bovine udder. It must be borne in mind, however, that all chronic cases of staphylococcal infection of the cow's udder do not develop into "actinomycosis". For, it appears that Minett [1937], although admitting that "in its commonest form the disease is chronic", developing slowly into diffuse induration of the gland, has, in none of the six cases studied, encountered the actino-body. Colebrook [1930] mentions a case of Staphylococcal "actinomycosis" in the elbow of a French soldier, which is perhaps the only case of its kind known in man.

From bovines Magnusson has obtained still another bacillus—*Corynebacterium pyogenes*—in pure culture in five cases of jaw-lesions and five times from polypoid growths developed as complications of wounds in the wall of the rumen and reticulum, which showed metastases in the liver, lungs and

diaphragm. The primary lesions as well as the metastatic ones were histologically identical with typical "actinomycosis". Magnusson mentions that Hulphers had obtained the same bacillus from nineteen out of sixty-seven jaw-lesions and Gunst [1927] from four out of seventeen cases. Experiments to produce the disease by inoculation of the pus-granules into healthy bovines were negative. The bacillus may, however, be looked upon as one capable of producing a chronic granuloma containing actino-bodies.

From what has been mentioned above it will be apparent that in diagnosing "actinomycosis" in animals the etiological agent should be the deciding factor if an expressive nomenclature is to be adopted. It has often been realised that the use of the comprehensive non-specific term "actinomycosis" leads to confusion. Thus loose statements are sometimes made that in actinomycosis of the tongue and jaw, the lymph glands are affected; the condition actually implied is actinobacillosis.

Like Lignieres and Spitz [1902], Colebrook [1930] suggested the term **actinophytosis** to embody all disease conditions wherein granules with actino-bodies are formed, irrespective of etiology. Thus he suggested terms such as actinophytosis due to *A. bovis* (**actinomycosis**), due to *A. lignieres* (**actinobacillosis**), due to *Staphylococcus pyogenes*, or due to *Corynebacterium pyogenes*. He also made a difference between actinomycosis due to *A. bovis*, and **paractinomycosis** (also an actinophytosis) due to filamentous micro-parasites other than *A. bovis*. He reserved the term streptothricosis for infections due to "filamentous organisms but showing no granules, or with granules with no clubs". Bosworth [1930] suggested the terms 'actinomycosis' due to *A. bovis*, 'actinobacillosis' due to *A. lignieres* and 'botryomycosis' due to *Staphylococcus pyogenes*. There should be no objection to the adoption of the former two terms but the expression 'botryomycosis' would lead to confusion. As it is, the term is inappropriate even in the case of the well-known equine malady. Considering the facts that the term has become well established for the equine disease, that it is suggestive of a mycotic infection, and that the disease may also occur in the equine udder [Hutyra and Marek, 1926] the term should not be adopted for designating "actinomycotic" mastitis in bovines. Davies [1935] has suggested the term '**actinococcosis**' which is expressive and at the same time homologous with the other two terms, actinomycosis and actinobacillosis. But unlike Colebrook, Davies has not taken into consideration the evidence adduced by Magnusson [1928] in favour of *C. pyogenes* being a potential actinobody-producer in chronic infections. Should the contention of Magnusson be substantiated in future we will be required to add another 'actino.....' to Davies' list.

It is a pity that Colebrook's suggestions have not met with general approval in spite of the fact that his nomenclature is open to further addition of actinophytoses as new etiological agents are discovered, and that the term 'actinophytosis' is a non-specific and yet an expressive one, and should only be used to designate conditions other than actinomycosis and actinobacillosis which terms should be specifically employed.

In the collection of this Institute are specimens which have been received from different parts of the country for microscopical examination and diagnosis. Although the material is only meagre it is sufficient to prove the existence of at least some of the actinophytoses in India. To add to the evidence, and for comparative study, some material was especially obtained by Mr. T. J. Hurley, M.R.C.V.S., D.V.S.M., I.V.S., (Offg. Veterinary Research Officer incharge of Pathology, Imperial Veterinary Research Institute, Mukteswar) from the collections of the Veterinary College, Madras. To Mr. Hurley and to the authorities of the Madras College, I am greatly indebted for the help. The following record of the result of examination of the various specimens is not intended to give a histological study of the materials, for this has already been done by many other workers [Bosworth, 1923 ; M'Fadyean, 1932]. It is intended to show how with the help of available staining methods the etiology of the actino-body can be ascertained with great success.

#### A. SPECIMEN 1357/1928

The material was received in May, 1928, in the form of two small pieces of tissue removed from the tongue of a six-year old country-bred, emaciated cow showing "swelling of the tongue with granulation tissue, difficulty in taking food; foaming saliva coming out from the mouth". The condition was suspected to be "actinomycosis".

The tissue on histological examination proved negative at first, but on examination of sections prepared later "beautiful actinomyces clubs" were demonstrated but the responsible organism was not determined. As the material was exhausted, the author had to be satisfied with re-examination of available stained sections. Fragments of an actino-body, surrounded by a zone of cellular reaction, were seen in each section. The entire lesion was microscopic, non-elevated and situated superficially, embedded in the markedly hypertrophied epithelial layer of the tongue (Plate XIX fig. I). The focus had an extremely ill-formed outer capsule.

On restraining some of the preparations, not Gram-positive but Gram-negative small bacilli were detected, the lesion resembling the one described later (Specimen 327A/1936, p. 283).

This case appears to be the first in the records of this Institute, which from the clinical and histological features can be interpreted as one of actino-bacillosis.

#### B. SPECIMEN 1557/1929

The information available in connection with this specimen is rather limited. The tissue was removed from the shoulder of an ox and carcinoma was suspected.

A diagnosis of "actinomycosis" was given in 1929. In freshly cut sections (1937), the tissue showed itself to be the product of connective-tissue reaction in the subcutis. In it were found microscopic pus-foci which faded out peripherally into the surrounding granulomatous tissue. The centre of

several foci revealed actino-bodies with clubs which were larger and more prominent than in actinomycosis (Plate XIX, fig. 2). In suitable sections, it was discovered that the minute central portion of the ray-colonies was occupied by a cluster of Gram-positive bacilli. Their exact identity could not be determined but such characters, as are usually associated with diphtheroids, were observed, e.g., uneven staining, filamentous and coccoid forms, and club-like swellings. Fortunately a preparation of *Corynebacterium pyogenes* in tissue was available for comparison which afforded strong suggestive evidence in favour of the view that the bacteria in the actino-bodies were diphtheroids.

In and around the pus-foci eosinophiles were very frequently seen but a careful search for worms or worm-ova, in sections and in caustic potash preparations, proved entirely negative. No foreign body giant-cells were seen. Of the skin, only the deeper layers of the epidermis were visible in certain sections but no carcinoma was detected.

The case was evidently one of actinophytosis due to a pleomorphic Gram-positive bacillus (? *Corynebacterium*) but not due to a *Streptothrix*.

#### C. SPECIMEN 1857/1931

The material, which unfortunately could not be traced, was received from the Principal, Madras Veterinary College. It belonged evidently to the buffalo, referred to in the Annual Report of the Civil Veterinary Department, Madras, 1927-1928. It may be recalled that a Gram-positive *Streptothrix* was observed in sections. Although all necessary information is not available with regard to this material, it is possible that the case was one of actinomycosis.

#### D. SPECIMEN 1862/1931

This specimen forms the subject of the paper presented by Datta [1933] to the Indian Science Congress in 1933. The material was received from the Superintendent, Sind and Rajputana, and consisted of "actinomycosis tumours removed from both parotid glands of a.....buffalo heifer". The tumours were situated "about three inches below the base of the ears" and "measured three inches in diameter". They were first noted as walnut-sized growths when the animal was about a year old and had grown to the size mentioned in six months at the end of which period they had begun to "emit a discharge" (fistula-formation) of the nature of stringy "thick inspissated pus". There was difficulty in deglutition and breathing. After removal of the tumours, iodine treatment was adopted and "the heifer showed marked improvement".

On first examination of the tumour in October, 1931, "actinomycosis" was confirmed except that no Gram-positive filaments were seen. Subsequent examination of sections made in August, 1932, and stained by Gram-Weigert revealed mycelial filaments inter-twined inside the "ray-fungus".



FIG. 1.  
Actinobacillosis of bovine tongue  
(1357×1928 ;  $\times 36$ )

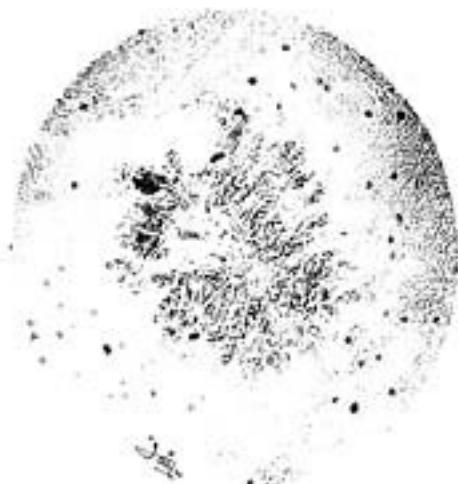


FIG. 2.  
Actinophytosis of bovine shoulder  
(1557×1929 ;  $\times 396$ )



FIG. 3.  
Actinobacillosis of parotid gland  
(1862×1931 ;  $\times 51$ )



FIG. 4.  
Actinobacillosis of parotid gland  
(1862×1931 ;  $\times 792$ )



FIG. 1.

Hill bull 749, showing Actinomycosis of the head

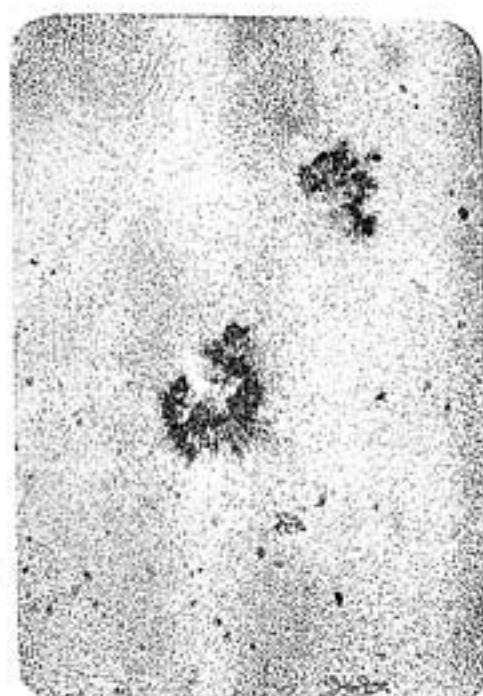


FIG. 2.

Colonies of Actinomyces in cheek nodule  
(175/1934;  $\times 81$ )

FIG. 3.

Actinomycotic lesion in the skin (175/1934;  $\times 40$ )

A re-examination of the material was undertaken this year (1937) and numerous sections cut and stained by different methods. Histologically, the tissue represented an advanced chronic lymphadenitis with formation of abundant connective tissue wherein several small actino-bodies, usually in groups, were seen (Plate XIX, fig. 3). These were surrounded by a varying amount of cell-detritus. By Gram-Weigert method of staining, no evidence of a Gram-positive organism was detected. By modified Ziehl-Neelsen method (using two per cent sulphuric acid instead of twenty-five per cent), however, a beautiful and instructive picture was obtained (Plate XIX, fig. 4). The peripheral clubs were well formed and numerous. No filaments of streptothrix were observed in the colonies. Instead, centrally to the zone of clubs, and rarely in the peripheral zone itself, numerous masses of (Gram-negative) short bacilli were seen which could be clearly made out in the smaller (<sup>†</sup> early) single colonies.

The results of the last examination agree with those of 1931 but cannot be reconciled with the observations recorded in 1932. The possibility of an actinomycetic infection is so remote as to be ruled out in view of the fact that actinomycosis does not spread by the lymphatics. I may here quote from Cope [1930] :—

"Concerning the conduct of the actinomycotic fungus in the tissues of the body, there are three points which are peculiar and characteristic. The first is the way in which the process advances almost always by contiguity of the tissue. Blood transmission occasionally occurs, as for example infection of the liver from the appendix through the portal veins, but usually the slow inflammation progresses by contiguity of tissue, often receding in one place and advancing in another. The second characteristic is the curious immunity of the lymph glands to the infection. I have never seen a lymphatic gland infected by Actinomycosis, and have never seen any authentic record of such a happening. This feature places it in sharp contrast not only with the actinobacillosis infection of cattle, but also with almost every other variety of infection both acute and chronic".

The clinical history and histological features including absence of Gram-positive filaments in this case are thus indicative of a certain actinobacillary infection. It is unfortunate that no information about the existence of disease in other organs was available.

#### E. SPECIMEN 175/1934

This material was collected locally from a clinical case in a Hill-Bull (No. from 749) while it was alive (Plate XX, fig. 1) and consisted of a nodular growth the right cheek, and a piece of skin. "Typical lesions of Actino" was the diagnosis given. Bone-lesion was suspected during life.

In this case it was unfortunate that no other observations were recorded. The animal was subsequently destroyed but no *post mortem* examination was conducted. The site of the removed skin was similarly not recorded, but was probably in the neighbourhood of the nodule.

On histological examination there could be no doubt about the actinomycotic nature of the lesions. In the cheek, the "nodule" consisted of an abscess, about 0·75 cm. in diameter, in the thickness of the cheek away from the outer surface. The focus consisted mainly of polymorphs and contained numerous actino-bodies in different stages of formation. The Gram-positive branching streptothrix could be easily demonstrated in the lesion (Plate XX, fig. 2). It showed occasional terminal swellings, and slightly uneven Gram-positive character.

In the skin the dermis showed signs of connective tissue proliferation (Plate XX, fig. 3) and contained a few pus-foci with actino-bodies due to the Gram-positive streptothrix.

#### F. SPECIMEN 245, B/1934

The material was from a twelve-months old, cocoanut-sized, inoperable tumour of the lower lip of a bullock from Sind. Actinomycosis was suspected and confirmed by the evidence of "a few actino-granules" and "numerous mycelial masses".

It was unfortunate that old sections of this material were not available and that the material had become exhausted except for pieces of what appeared to be the reacting well-formed connective tissue. Repeated attempts to verify the diagnosis by making fresh sections proved of little value as nothing but the old granulomatous tissue was encountered in sections.

Yet, by the situation of the lesion [*Cf.* Specimens 175/1934 (p. 279) and 194/1937 (p. 284)] and by the diagnosis already given in 1934, there seems little doubt that the case was one of actinomycosis.

In connection with the formation of abundant connective tissue in this case, the quotation from Cope (1930) may be continued :

"..... Thirdly, everyone who has seen much of actinomycosis must have pondered over the remarkable reaction which the fungus causes in the connective tissues. I think it was Unna who pointed out that here we have an almost unique example of tissue reaction at a distance, for the changes may take place at a considerable distance from the place where the fungus is situated, and it is possible to cut many sections of the curiously hard gristly fibrous tissue, without finding any filaments of the parasite. This connective tissue reaction differs considerably from that found in tuberculosis and syphilis not only in the relative proportion of the various kinds of cells found in it, but also in the fact that the blood vessels are not obliterated. Exactly how the fungus causes the reaction is unknown".

#### G. SPECIMEN 192/1936

This specimen consisted of a piece of tumour from the nostril of a buffalo from the Civil Veterinary Department, Agra, U. P., presumably suspected for Schistosomal nasal granuloma. No other information was available in this case. A preliminary diagnosis of "actinomycosis" was given, but as no Gram-positive micro-organism was seen in sections a detailed study of the specimen was continued. At the end, the growth was diagnosed as actinobacillosis,

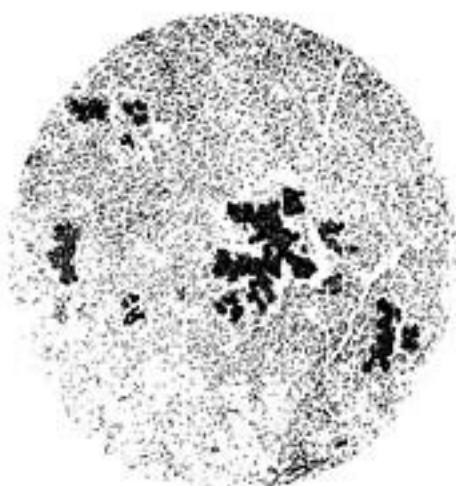


FIG. 1.  
Actinobacillary nasal growth (192/1936 ;  $\times 67$ )



FIG. 2.  
Actinobacillary nasal growth  
(192/1936 ;  $\times 720$ )



FIG. 3.  
Villous growths on the heart  
(296/1936 ;  $\times 18$ )

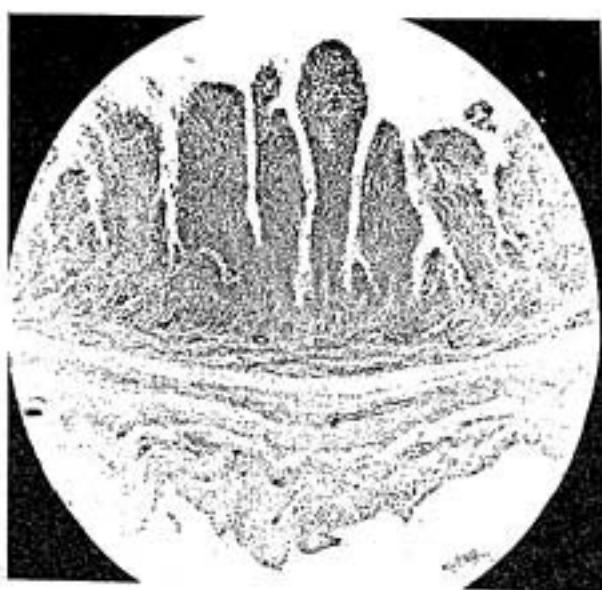


FIG. 1.

Villous growths on the pericardium (296/1936;  $\times 32$ )



FIG. 2.

Pericardium. Streptothrix colony  
(296/1936;  $\times 50$ )



FIG. 3.

Pericardium. Streptothrix colony  
(296/1936;  $\times 211$ )

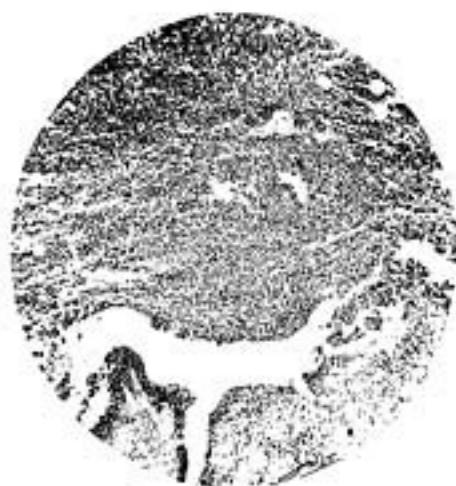


FIG. 4.

Pericardium, showing Streptothrix colony and  
worm-eggs (296/1936;  $\times 50$ )

The tumour consisted of a highly cellular granulomatous tissue under the ciliated nasal mucous membrane with numerous foci of cellular reaction, containing polymorphs and showing occasional giant-cell formations. In these foci were seen, as a rule, groups of small, often apparently confluent actino-bodies (Plate XXI, fig. 1) with well-formed clubs. Again, no Gram-positive mycelium was seen, but by modified Ziehl-Neelsen method of staining masses of (Gram-negative) bacilli (Plate XXI, fig. 2) were detected amongst or just under the clubs except in the smallest (? early) colonies, where they were situated in the centre of the actino-body. The centre of the large (?) older) colonies was left as an unstained more or less homogeneous mass.

This specimen with which Specimen 1862/1931 (p. 278) affords a very remarkable comparison, offers good material for the study of typical actinobacillosis. Enquiries made for obtaining more information and material, especially for cultural examination, were unfortunately disappointing, the animal having died during the interim.

#### H. SPECIMEN 296/1936

This material, the only one of its kind in the collection of the Institute, was received from Mr. Mutappa, Veterinary Assistant Surgeon, Ponampet, Mercara, to whom I am indebted for also furnishing a complete history of the case. The patient was a cross-bred spaniel of about two years which after a course of treatment for suspected distemper came under the observations of Mr. Mutappa on the 6th October 1936. High fever, dyspnoea, and an anxious look were noticed. The animal usually stood with the elbows turned out. The pulse was weak and irregular. Daily injections of Omnidin did little good and on the third day the animal was much worse, with laborious breathing and an almost inaudible heart-action. The patient died on the 9th October 1936. On autopsy the "thoracic cavity was found to be filled with serosanguineous fluid. The pericardial sac was also highly enlarged with the sero-sanguineous fluid. Round about the pericardium, there were fibrinous growths. The lungs were normal. No parasites either in the lungs or the intestines were detected by me".

The material submitted for examination was heart muscle and pericardium in formalin.

With the naked eye, nothing beyond a fine villous proliferation on the outer surface of the heart (epicardium) and on the inner surface of the pericardial sac could be seen. On histological examination the suspicion of a neoplastic disease was at once proven to be mistaken. The fine velvety appearance of the tissue was revealed to be a highly vascular granulomatous reaction (Plate XXI, fig. 3, and Plate XXII, fig. 1).

In the epicardial lesion no actino-bodies were seen but fragments of a Gram-positive micro-organism in the form of fine branching filaments and coccoid forms, some contained within wandering cells, were observed.

The pericardium was unevenly but markedly thickened, and showed the disease in a more severe form, the entire organ being involved and more or less thickly covered with papilliform growths. The connective tissue reaction was severe and in the form of a highly vascular granulomatous tissue, as before, wherein foci of wandering cells, by no means rich in polymorphs, were seen. In these foci thickly felted masses of a Gram-positive *Streptothrix* (Plate XXII, figs. 2 and 3) could easily be detected. There was, however, no definite club-formation, although a slight swelling of the peripheral ends of some of the filaments was suggested. The filaments showed an uneven Gram-positive character and the cell-collection around the ray-colony was at places very scanty.

A most remarkable feature of the pericardiac lesion was the presence, in the granulomatous tissue, of eggs (Plate XXII, fig. 4 and Plate XXIII, fig. 1) which in caustic potash preparations were observed to be operculated. No worms, or evidence of eosinophilia was noticed. The eggs were, in all probability, of a trematode, and in view of the thoracic situation of the lesions and of the reported existence of the lung-fluke *Paragonimus westermani* from dogs in South India, the ova, by their size, may well have been of this species, although no worms were detected by Mr. Mutappa at autopsy. How these eggs came to be situated in the lesions and what part, if any, they played in the causation of the "actinomycotic" disease are questions which cannot be readily answered.

Although *Streptothrix* infection in canines is definitely rare, various authors have, from time to time, observed its occurrence in other parts of the world, and some of them have expressed the identity of the causative organism with *A. bovis*. Vachetta [1882] was the first to detect "actinomycosis" of the jaw of a dog. Rivolta [1884] described a purulent pleurisy in a dog due to an unbranched leptothrix type of organism. Rabe [1888] described three cases of "actinomycosis" of the dog; in two there was suppurative phlegmon, and in the third a blood-tinged purulent peritonitis was observed. A filamentous organism, *Cladothrix canis* (Rabe), was seen in the actino-bodies. Rabe [1888] succeeded in transmitting the disease to a dog by inoculation of pus from the third case, and recovered the organism. Hartl [1901] met with one case of "actinomycotic" tumour in the mediastinum and thoracic wall. Bahr [1904] described "actinomycosis" of the dog and cultivated the causative streptothrix. Schnelle [1929] described the case of a dog, similar to that of Mr. Mutappa, but wherein abscesses appeared on the skin which showed on autopsy an effusive pleurisy with reddish cloudy exudate, granulations on the pleurae, broncho-pneumonia with consolidation, and greyish nodules in the lungs throughout. Microscopically, *Streptothrix canis* was detected. Feldman and Mann [1932] described an extensive granulomatous condition of the sub-mucosa of the dog's stomach with typical actino-bodies with clubs but without a demonstrable central Gram-positive filamentous mass either in sections, or in culture, or by animal inoculation. Histologically, their case resembled to some extent the one encountered by the author in a bovine [Spec. 1557/1929 (p. 277.)]. Sheather [1932] reported a finely divided papilloma-like growth removed from the thoracic cavity of a dog, which histologically revealed the

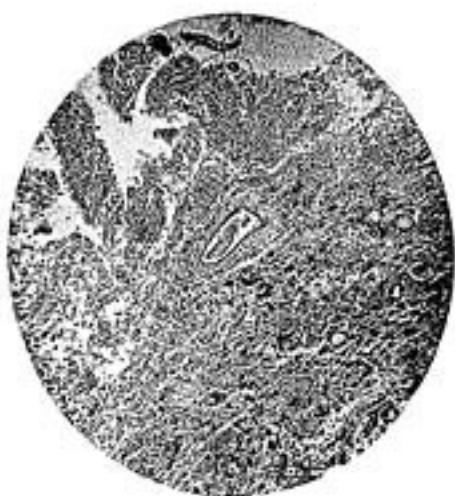


FIG. 1.

Pericardium showing worm-eggs  
(296/1936 ;  $\times 50$ )



FIG. 2.

Actinomycosis of bovine tongue  
(327-A/1936 ;  $\times 36$ )



FIG. 3.

Actinomycosis of gum of cow  
(191/1937 ;  $\times 81$ )

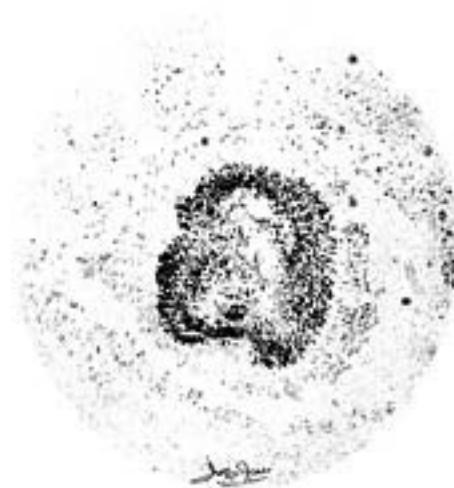


FIG. 1.

Actinomycosis of gum of cow  
(194/1937 :  $\times 135$ )



FIG. 2.

Actinomycosis of bovine udder  
(194/1937 :  $\times 81$ )



FIG. 3.

Actinomycosis of bovine udder  
(194/1937 :  $\times 576$ )

picture of typical granules without clubs. Baudet [1934] studied three cases of cutaneous "actinomycosis" of the dog wherein the blood-stained granular pus showed Gram-positive branched and unbranched filaments with terminal swellings but no clubs. In culture the organism corresponded with *Cohni-streptothrix* [Pinoy, 1911, Syn. *Streptothrix*, Cohn, 1875] *israeli* [Syn. *Actinomyces bovis*, Harz 1877]. Baudet named it *Cohnistreptothrix canis*. Fethers [1934] described a case of "actinomycosis" of the skin of the dog, which led to purulent peritonitis with involvement of the abdominal wall and liver. Microscopically a Gram-positive *Streptothrix* with rare clubs was seen.

In India, too, the condition in the dog is very rare, the meagre information about its occurrence being only in the Annual Report of the Civil Veterinary Department, Madras (1934-1935).

It will be seen that the evidence is in support of the occurrence of *Streptothrix* infection in the dog. By comparison with similar cases of other authors, Rivolta's case would appear to be one of actinomycotic infection. Although in some cases the available information does not render classification on Colebrook's lines possible, the great majority of them do not show definite club-formation and evidently belong to the group of Streptothricoses. Until more work is done regarding the causative organism (s) of *Streptothrix* infections in canines, the organisms named *C. canis* (Rabe) and *C. canis* (Baudet) may be conveniently brought under the more generally adopted generic name, *Actinomyces*.

The existence of *A. canis* is in fact recognized in modern veterinary textbooks which describe it as a specific, chromogenic organism of which both aerobic and anaerobic strains exist. The parasite is pathogenic to mice, rabbits and dogs—certain strains to calves and horses—and infects the dog through skin-wounds or through the respiratory tract producing, in rare cases, involvement of the regional lymphatic glands and of the internal organs and joints.

#### I. SPECIMEN 327A/1936

This specimen was removed *post mortem* from the tongue of a Hill-Bull (No. 368) which died of experimental rinderpest at the Imperial Veterinary Research Institute, Mukteswar. The lesions consisted of five fair-sized papillomatous growths on the prominence of the tongue. They were attached by a broad base except in one case where the lesion had a stalk. A benign neoplasm was suspected and so no other observations were made.

Histological examination of the fixed tissue revealed the growths to be due to a Gram-negative bacillus. At the base of some of the polypoid growths, as well as higher up, foci of wandering cells (Plate XXIII, fig. 2) containing actinobodies were seen. The growths were covered with the epithelium of the tongue and around the foci reacting connective tissue was observed. No Gram-positive mycelium was detected in the lesion. The muscles of the tongue showed scanty sarcocysts.

The lesions were interpreted as those of actinobacillosis.

## J. SPECIMEN 194/1937

This was received by special request, from the Veterinary College, Madras. It consisted of—

(a) a scirrrous lesion of typical actinomycosis of the gum of a cow (Plate XXIII, fig. 3, and Plate XXIV, fig. 1), and

(b) what was described as "actinomycosis" of the udder of a buffalo.

The latter tissue showed, in sections, a well-marked granulomatous change and the presence of scattered actino-bodies surrounded by areas of pus-cells (Plate XXIV, fig. 2). Club-formation was prominent. By Gram-Weigert staining method no streptothrix was discovered, but the central portion of the ray-colonies showed what appeared to be a Gram-positive, pleomorphic micro-organism. There were tortuous, (unevenly) thick, mycelium-like branching filaments and deeply staining small and large coccoid forms (Plate XXIV, fig. 3). It was impossible to determine, even grossly, the identity of the parasite, but it must be admitted that the case was one of actinophytosis not due to *Actinomyces*, *Actinobacillus*, *Staphylococcus* or *Corynebacterium*. It is possible that the organism was a mould.

No other specimens of "actinomycosis" could be traced in the collection of this Institute but the above material is sufficient to indicate the existence in India of at least three conditions, viz., actinomycosis and actinobacillosis in the bovine and streptothricosis in the dog. It is hoped that the need for cultural examination of relevant material will be realised because without this a systematic investigation of "actinomycotic" conditions is not likely to progress.

## GENERAL REMARKS AND DISCUSSION

We may now broadly recapitulate the position with regard to "actinomycotic" infections in animals in general, for, as every veterinarian is well aware, not only bovines and dogs but other animals also suffer from such infections. The horse is known to be susceptible to infection by *Actinomyces*, which may be, at least in some cases, of the nature of true actinomycosis [Perroncito, 1887; Baranski, 1888]. Several cases of the disease in the horse have been recorded [Johns, 1885; Baranski, 1888; Perroncito, 1890; M'Fadyean, 1888; Klemm, 1889; Dean, 1899]. The organs reported to be affected include the submaxillary lymph glands [Baranski, 1888; Dean, 1899], spermatic cord [M'Fadyean, 1888] and internal organs (following castration), and femur [Perroncito, 1890]. "Wooden tongue" of uncertain etiology is also mentioned in some cases [Zschokke, 1888; M'Fadyean, 1889].

The existence of *Actinomyces equi* is recognized, but the information on the subject is so scanty as to make further research seem highly necessary.

From a case of pseudotuberculous pulmonary lesions in a camel, Mason [1919] isolated an aerobic streptothrix and named it *S. (syn. Actinomyces) camelii*. The disease was a streptothricosis.

From swine a large number of cases of "actinomycosis" have been described since the first case of Johne in 1879. The most common lesions are those found in the udder, particularly in old sows [Magnusson, 1928]. Although other sites of infection have been described, e.g., pharyngeal region, cervical or dorsal vertebrae, lungs, and substance of the skeletal muscle [M'Fadyean, 1889], it seems probable that not all take the form of actinophytosis. The lung lesions described by Albiston [1921] were, however, of true actinomycosis.

Magnusson failed, in his extensive work, to encounter in pigs anything comparable with the bovine actinobacillosis of the organs of the head, the lesions being exclusively in the udder, caused by two types of *A. bovis* and by *Staphylococcus pyogenes*. He has singularly failed to meet with a case of actinobacillosis, although according to Griffith [1931], the bacillus is known to infect porcine udders. Experimental inoculations in pigs, using the *Streptothrix* and the *Staphylococcus* from actual lesions, have been successful [Magnusson, 1928].

In calcareous deposits in pig's muscles, an *Actinomyces*—*A. musculorum*—has been seen [Dodge, 1936]. It has not been cultivated.

In bovines actinophytosis may appear in an epizootic form [Lignieres, and Spitz, 1902; Stenon, 1891; Mattinson, 1932]. All the four types of actinophytosis viz., those due to *A. bovis*, *A. lignieresii*, *Staphylococcus pyogenes* and *Corynebacterium pyogenes* are known, the udder lesions being always staphylococcal in origin. From the literature it is evident that the disease affecting the soft organs or the bones of the head, or the skin, internal organs and the serous membranes [Bosworth, 1930], where the lymph glands are also infected, is always due to *Actinobacillus*; whereas, in actinomycosis proper which affects the bone or the neighbouring soft tissues, there is no involvement of the glands. According to Delepine [1890] and Bosworth [1923] the glands may be enlarged and denser due, not to infection by *A. bovis* but due to irritants produced by it in the lesions. Contrary to what is mentioned in some text-books actinomycosis of the tongue does not exist; the causative organism to be isolated from "wooden tongue" has always been *A. lignieresii*, never *A. bovis* [M'Fadyean, 1932]. In the words of Magnusson, "from what we know, there is simply no actinomycosis of the tongue in cattle, the affection being always actinobacillosis of the tongue."

Although "lumpy jaw" is, as a rule, an actinomycosis [Davies, 1932; Sedlmeier, 1936] cases are not uncommon where the condition has been caused by *Actinobacillus* [Bosworth, 1923; Magnusson, 1928]. In spite of a few exceptional reports of infection of lymph glands by *A. bovis* [Weighton, 1932; Thompson, 1933], it is generally accepted that in bovine actinomycosis "the lesions are for the most purely local and if any dissemination takes place it is by way of the blood channels. A lymphogenic spreading hardly ever occurs. Lymphadenitis does not occur. If the lymphatic glands are involved we may be sure that the causative agent is not *Streptothrix israeli*, but *A. lignieresii*" [Magnusson, 1928]. Blood transmission sometimes occurs in man [see Cope,

1930 and, in view of the above statement of Magnusson, perhaps in rare cases in animals. In this connection the report, by Awrorow [1932], of bovine renal actinomycosis may be of interest.

With material from actual cases of infection by *A. bovis*, the disease has been successfully transmitted to cattle [Magnusson, 1928], but not to dog, sheep, goat and horse. Similar success has attended experiments wherein *Actinobacillus* from natural cases was inoculated subcutaneously into cattle, but only rarely does a strain of the bacillus prove intraperitoneally pathogenic to guinea-pigs. Hulphers [1923] has produced staphylococcal "actinomycotic" mastitis in a cow experimentally, using cultures from a natural case; but, Magnusson was not successful in similar experiments of his own.

Research workers [Lignieres and Spitz, 1902; Bosworth, 1930; Magnusson, 1928; Griffith, 1931] claim that diagnosis of actinobacillosis can be made by use of the agglutination test. Recently, however, Thompson [1933] has pointed out difficulties in utilizing the test for cattle as considerable antigenic variation exists between strains of *Actinobacillus*. In New Zealand [1934], the test is reported to be giving useful results.

Attempts to diagnose actinomycosis in the living patient by serological methods have not been successful. Colebrook [1920] states that in man the serum of a heavily infected case will agglutinate *A. bovis*. This test has been useful in ascertaining the presence of more than one serological type of the organism but does not appear to have been generally adopted in clinical practice. Attempts to develop an intradermal allergic test in man have given inconstant results. The reactions obtained by Adant and Spehl [1935] are alleged to have been indicative of a mycotic, not necessarily actinomycotic infection. Mathieson, Harrison, Hammond and Henrici [1935] have found that normal persons often showed a more marked reaction to the test than did actinomycosis-patients [Mark and Auerbach, 1937].

The finding of Corynebacterial actinophytosis by Magnusson [1928], Hulphers [1923] and Gunst [1927] in jaw-lesions and in post-traumatic polypoid growths in the rumen and reticulum of bovines has not been universally recognised, perhaps on grounds of insufficient data. It appears that this disease-condition should be investigated further with the object of eliminating the possibility of a mixed infection in every case encountered. Cultural and biological tests by Magnusson [1928], however, tend to exclude this possibility.

Gillain [1932] has recently recorded a case of actinomycosis of the superior maxilla of a bovine due to *A. breviora*, but the study of the organism is incomplete.

Actinophytosis is also known to occur in sheep. It affects the jaw, lips, tongue, and lungs. The common causative organism appears to be *Actinobacillus*.

*Actinomyces caprae*, an aerobic species, has been recorded from pseudotuberculous lesions in goat's lungs in 1899 [R. St. John-Brooks, 1931].

Edington [1934] has isolated *A. bovis* from a subcutaneous sinus of a cat. The sinus was discharging pus containing granules showing the *Streptothrix*.

Cases of *Streptothrix* infection, not amounting to actinophytosis, are also known to occur in the fowl. *Actinomyces tissicus* (aerobic) has been isolated from tumours in the abdominal cavity. The only case reported from a fowl in India is that of streptothricosis of the cloacal region (Annual Report of the Civil Veterinary Department, Madras, 1933-34).

Amongst wild animals, too, infection by *Streptothrix* is by no means uncommon. " Moody has described lesions, clearly actinomycotic in the bones of a fossil rhinoceros. The disease is known to occur in deer and moose, sometimes in epidemic form " [Henrici, 1930]. Fox [1923] mentioned the occurrence of actinomycosis in American Tapirs and has isolated an aerobic species, *Actinomyces (Nocardia\*) macrodipodidarum*, from lesions resembling " lumpy jaw " in Kangaroos. The Kangaroo disease has been described as a streptothricosis causing swellings and ulcerations about the lips, tongue and teeth.

It has already been stated elsewhere that the aerobic *Streptothrix* of Bostroem is now generally regarded as a mere saprophyte of no etiological significance and that actinomycosis in man and animals is caused by the Israel type of organism viz., *A. bovis*. There is, however, no definite information as to the pathogenesis of the disease. The findings of Lord [1910] that *A. bovis* is normally found in the mouth cavity (and alimentary tract, Naeslund, 1931) of normal individuals and particularly around carious teeth, and that local injuries assist in setting up the disease, have found general support [Cope, 1930; Davies, 1932; Naeslund, 1931; Mark and Auerbach, 1937]. It is thought that in some cases of actinomycosis of the lungs the organism may have been conveyed from the mouth by aspiration. The idea that *A. bovis* lives in the soil and on cereal plants and infects through contaminated food (straw and grain) has now been practically abandoned.

Once the organism has established the infection in the tissues, further progress of the disease is only a question of time. Pus-formation by accumulation of cell detritus around the actino-body occurs as a result of tissue reaction in which neutrophils, lymphocytes, fibroblasts and endothelial cells take part. The microscopic sulphur granules are thus formed. (It appears to the author that granules need not be macroscopic in all actinophytoses). As the ray-colony grows (? terminal) portions of the mycelium are said to resolve themselves into minute coccoid bodies, comparable, according to some [M'Fadyean, 1889; Bosworth, 1923], to conidia, which are liberated, as it were, to be engulfed, as also are detached fragments of the mycelium itself, by phagocytes. These cells may either digest the infective agent or may help to convey it to

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\*The generic term *Nocardia* is used by Wright [1905] and his followers to indicate the aerobic saprophytic actinomycetes, some of which may be pathogenic.

other neighbouring areas and thus start a fresh focus of infection. Not rarely these coccoid bodies and even mycelial filaments may be disseminated without the intermediation of phagocytes [M'Fadyean, 1889; Delepine, 1890].

The significance of the association, in human actinomycosis, of *Bacillus actinomyctem comitans* is not known. The bacillus was first described by Klinger in 1912 and has subsequently been encountered by others. It is a small, Gram-negative, pleomorphic, non-motile, non-sporulating, non-encapsulated organism and a facultative anaerobe, fatal intravenously to rabbits and intraperitoneally to guinea-pigs.

The formation of clubs at the periphery of the actino-body is still a matter of dispute. It is apparent from literature on the subject that clubs are in many cases, a result of tissue reaction [Meyer and Mayer, 1927]. In actinomycosis, however, they are said to represent parts derived from the causal organism. They are interpreted as degenerated mycelial ends [M'Fadyean, 1889]; or are explained, by many workers, to be formed by the thickening of the mycelial capsule [Delepine, 1890; Bostroem, 1890]. Others regard them as organs of fructification giving rise by budding to spores which help to spread the infection to fresh foci [Crookshank, 1888; Gibson, 1934]. Brumpt [1906] interprets them as the youngest members of the infective agent supplying nourishment to the older and more centrally situated members, the mycelia.

A brief note on the systematic position of *Actinomycetes* may be made here. For want of an accurate knowledge of morphology, the claim on this group is divided between bacteriologists and mycologists. The former base their arguments mainly on the unusually slender mycelium (0.2 to 1.0  $\mu$ ) and on the production of bacilliform asexual reproductive elements (arthrospores) of the same thickness as the mycelium, and include the organisms under the Schizomycetes; whereas, the latter emphasize the formation of spores similar to that of conidia in the Fungi Imperfecti (Hyphomycetes), and on the markedly low heat resistance of these spores. Still others regard them as intermediate forms between bacteria and fungi, or as a parent group from which sprang the fungi and certain bacteria viz., *Mycobacteria* and *Corynebacteria*. Some authors [Magnusson, 1928; Haupt and Zeki, 1933; and Klimmer, 1934] have suggested affinities of *A. bovis* with the *Corynebacteria*; a few others [Grootenhuis, 1934] have put forward a plea in favour of the Mycobacterial nature of *Actinomycetes*, and as in the case of *Mycobacterium tuberculosis* evidence of a filtrable form of *A. bovis* has been recently put forth by Sartory, Sartory and Meyer [1933].

The position of *Actinobacillus* amongst the bacteria is not disputed but although it is generally considered to belong to the family Actinomycetaceae, Beaver and Thompson [1930] have suggested its place alongside *Pfeifferella*.

It will be realised from this paper that the position with regard to "actinomycotic" diseases is very unsatisfactory, not only in India but also in other countries whence, in spite of intensive researches, conflicting statements are not infrequently reported regarding various aspects of the subject. The problem is intensified considerably for the veterinarian by the fact that not one

but a number of etiological agents are responsible for producing such closely similar lesions that definite etiological diagnosis can be arrived at only after cultural and microscopical examination of the material. The following statement of Henrici (1930), made in connection with actinomycosis only, will suffice to illustrate this point :—

" One of the most remarkable facts about Actinomycosis is the volume of literature which has been written about it, and the small amount of information contained therein. In spite of the fact that the disease has been well known for half a century, and probably more than a thousand papers have been published on it, we are still somewhat uncertain as to the mode of infection and transmission, as to whether the disease can be contracted by man from cattle, for instance, and still a little in doubt as to the nature of the causative organism, whether one or several species of actinomyces are concerned."

#### SUMMARY

The subject of "actinomycosis" is discussed especially from the veterinary point of view, and a case made out for the adoption of a suitable nomenclature.

A few cases from the bullock, buffalo and dog are described from India in proof of the existence of the disease in this country.

The need for research especially from the cultural and biological standpoints is emphasized.

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ON THE RELATIONSHIP BETWEEN 'LAHORE CANINE  
FEVER' AND 'TICK FEVER' OF DOGS DUE TO  
*P. GIBSONI* INFECTION, WITH OBSERVA-  
TIONS ON THEIR PATHOLOGY AND  
HAEMOCYTOLOGY

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(With Plates XXV—XXVIII and seven charts)

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INTRODUCTION

THE tenure of life of dogs in Lahore is short. Very few well-bred or imported dogs reach maturity. The chief reason for this loss of dogs is a widely prevalent fever, popularly called 'Lahore Canine Fever', about which many opinions have been expressed and many treatments expounded without materially affecting the incidence or mortality. There are good reasons for believing that this particular variety of fever finds an expression in places elsewhere than in the Punjab, and it would appear that the disease is universal in Northern India, if it does not have a wider distribution. It is believed that dogs are subject to the disease in all parts of India and mention of a similar one has been made from Burma, Ceylon and Assam.

It is difficult to place on record an actual census of incidence and mortality but the recital of the fate overtaking the writer's dogs is so representative of other people's experience that it will serve as an indication of the frequency of the disease. The writer lost five dogs within a period of three years, all pedigree dogs born in India of imported parents, and all victims of the disease within two years of birth. The only survivor was a pure-bred fox-terrier, which recovered after a protracted illness, and, as the writer's experience is not exceptional, one can obtain a fairly accurate estimate of the general incidence and mortality.

'Lahore Canine Fever' affects all ages and breeds of dogs. Imported dogs suffer most severely. They are practically certain victims sooner or later. It is very doubtful if this type survives, on an average, for more than a few years.

Dogs bred in India of imported parents are equally susceptible. The pariah is by no means refractory to the disease, but as cases are so infrequently encountered in these animals, it must be assumed that they possess a marked degree of natural immunity. This observation is further enhanced when the

degree of resistance exhibited by pariah crosses is noted. The resistance to infection and the likelihood of recovery can be computed, within reasonable limits, on the basis of the percentage of pariah blood in the cross. There are strong grounds for suspicion, therefore, that pariahs are the natural sources of infection.

A definite seasonal incidence exists in 'Lahore Canine Fever.' The smaller incidence occurs during the beginning of the hot season, i.e., from the middle of March to the end of May and the greater towards the end of the hot weather, i.e., from the middle of September to the first or second week in November. Very few cases have been encountered in the real hot season.

This interesting observation immediately afforded a clue in the investigation. The periods noted synchronise with the two tick seasons, and it very quickly became obvious that ticks were of important significance in the spread of the disease. The first question that naturally presented itself seemed legitimate—Is this disease 'tick fever'? The answer, on available evidence, appeared negative. Routine examination of blood films was consistently negative in the great majority of cases. As this constituted, at the time, the sole available criterion on which to formulate a diagnosis, a negative diagnosis of 'tick fever' was made and the etiology sought elsewhere.

It must be mentioned, however, that in a few cases, presenting identical clinical symptoms, a diagnosis of 'tick fever' (*P. gibsoni* infection) was made. This finding immediately led to the question—Are all cases of clinically comparable canine fever referable to one etiological factor, *P. gibsoni*, and is the natural deduction tenable that blood film examinations of suspected cases are an unreliable means of determination of infection?

The main purpose of this paper is to produce evidence that 'Lahore Canine Fever' is, in actual fact, 'tick fever' (*P. gibsoni* infection), in a form that can only be diagnosed with extreme difficulty, and to show that the accepted methods of diagnosis by routine blood film examination are unreliable.

Mention must be made of the earlier experimental work and attempts to reproduce the disease in young pariah pups by blood transmission from diseased dogs. These experiments were unsuccessful, owing to the relative insusceptibility of such experimental subjects, as has already been stated. The injection of even large quantities of blood failed to reproduce the disease and it was presumed therefore that:—

- (a) the disease is not inoculable, and
- (b) the disease is not 'tick fever'.

That this was a premature and unwarrantable presumption is suggested on the grounds that, in a true *P. gibsoni* infection the blood is not uniformly infective. Although the animal from which the blood is taken is known to be a positive clinical case, there are phases of the disease when parasites are certainly not present in the blood, (See Charts) and the failure to transmit infection to susceptible animals—and earlier experiments were done on pariah dogs—can easily be explained. Even in direct transmissions with blood known to contain

*P. gibsoni*, a successful infection—as evidenced by the production of a disease of similar syndrome—may be characterised by the complete absence of *P. gibsoni* in the circulating blood during the course of the illness (See Charts Nos. 2, 5 and 7).

Mention must also be made of the efforts to prove and substantiate early claims [Manifold, 1928] that cases of canine fever, occasionally in enzootic form, could be attributed to infection with organisms of the *colon-typhoid* group, strains of *S. typhosus* in particular. Standard authorities unanimously agree that *S. typhosus* is not pathogenic for the dog and the writer's attempts to induce the disease artificially in dogs by inoculation of selected strains of *S. typhosus* of human origin is in accordance with the views of authorities on the subject. It is not clear, however, that Manifold was investigating the same type of canine fever that the writer has under review.

In 1929, Osmond Bodman (unpublished work) isolated a *Salmonella* strain (probably the same organism that is now recognised as *S. bareilly*, Bridges and Scott, 1931) which he considered responsible for a disease of dogs and which he designated 'Indian Tick Typhus'. There appears no doubt from a study of the existing records that Bodman was investigating a disease of clinical characters precisely comparable with those of 'Lahore Canine Fever'. It is important to note that Bodman incriminated the common dog tick as the vector of the disease.

Later, Cooper (unpublished work) examined several cases of canine fever which he considered identical with those described by Bodman and which he designated 'Canine Enteric Fever'. He isolated from several of these cases certain *Salmonella* strains, no two of which were identical, conforming superficially in type species to *S. enteriditis*, *S. aertrycke*, *S. paratyphosus B.*, *S. newport* and *S. flexneri*. He did not proceed sufficiently far with the work, however, to classify these organisms accurately and prove their authenticity on their antigenic structure. It must be assumed, from the variety of species isolated, that the organisms were not of authentic species, and were, in all probability, not the cause of the disease. The presumption is justified when the fact is recorded that the sera of the affected dogs failed to show homologous agglutinins against the types isolated.

A certain amount of work has been published on *B. aertrycke* infection of the dog and it must be mentioned that the clinical syndrome of the disease under investigation and that of a true *aertrycke* infection bear no comparison. In 1931, the writer had an opportunity of testing the pathogenicity of Cooper's *Salmonella* strains on selected experimental dogs. With none of them could disease in any form be induced.

Datta [1935] isolated, from an outbreak of canine fever in certain kennels in the United Provinces, an organism bearing superficial resemblance to *S. typhosus*. (This organism has since been typed as *Bacillus pseudotuberculosis rodentium*).

This sums up the efforts made to prove a bacterial etiology in this particular form of canine fever. The evidence is unconvincing. The writer has on several occasions isolated *Salmonella*-like organisms from the faeces of dogs affected with 'Lahore Canine Fever'. He has equally frequently isolated similar types of organism from healthy dogs and has refused to consider their potential pathogenicity, in that the serum of affected dogs failed to betray the slightest trace of agglutination and their inoculation by various routes yielded consistently negative results.

Finally, the study of the temperature charts of the affected dogs and their clinical syndrome in comparison with the known syndrome and pyrexial phase of *Salmonella* infections in the human subject, enhanced the uncertainty of accepting a bacterial etiology for this affection of dogs. The writer is further convinced of the insupportability of the claims to establish a bacterial etiology as he had an opportunity of subjecting certain of the fever cases, from which the *Salmonella*-like strains were isolated, to *post mortem* examination. The findings were identical, both on the pathological and histological grounds, with those present in 'Lahore Canine Fever.'

#### SYMPTOMATOLOGY

The disease is of sudden onset. Without any warning, a dog which had appeared perfectly normal a few hours previously, is found dull and dejected. The temperature is discovered to be higher than the physical condition would seem to indicate and is within the range of 104°—106°F. The usual concomitant features of fever are present. The degree of dejection which is present, the 'tucked up' appearance, the acute epigastric pain evinced, the occasional vomiting of a bile-stained fluid, are all typical. A peculiar symptom, and one frequently present, is a thin serous discharge, occasionally blood-tinged, from one or both nostrils. This nose may become partially occluded when this exudate dries or becomes thicker and the increased difficulty of respiration, the rapid breathing consequent on high fever, may lead the unwary observer to a fallacious diagnosis of pneumonia, and when, as not infrequently happens, an exanthematous eruption occurs in the inside aspect of the thighs, a mistaken diagnosis of distemper may be made.

A second peculiar symptom which is worthy of notice, is a slight puffy swelling in the mandibular space.

The fever, in spite of symptomatic treatment, undergoes little if any remission until the fifth to the seventh day, when it may suddenly drop to 102°F., within which range it may remain for several days. (It should be noted here that the normal temperature of the healthy adult dog in India is 100°F. and not 101—101·5°F. as commonly stated.)

The remission of fever is accompanied by a transient occasionally severe diarrhoea, the faeces being orange in colour and acrid in odour. The urine, which is darker than normal, contains traces of albumen. Haemoglobinuria is absent. The gums at their dental margins are angry, red and swollen and a typical mouth foetor is present.

The diagnosis of the case at this stage is frequently given, although wrongly, as acute gastro-enteritis and a superficial evaluation of the symptoms would seem to support this view. The temporary alleviation of fever is succeeded by an abrupt rise, possibly up to 106°F, and the temperature fluctuates between 103—105·5°F, for several days. If the dog, which is now critically ill, survives this crisis, the temperature drops fairly rapidly to a range within 102—104°F, and within ten to fourteen days, the temperature swings steadily between 101—103·5°F, with an occasional sharp paroxysmal rise of no great duration. The critical phase of the disease has abated, leaving the animal exhausted and severely debilitated. Superficial signs of anaemia, evidenced by pallor of the visible mucous membranes, are seen. The faeces are frequently black in colour at this stage of the illness. A distinct systolic thrill indicates an inestimable involvement of the myocardium. From this stage to the termination of the disease, a fluctuating erratic temperature is exhibited. For several days, the temperature may be practically normal. The dog appears brighter and an encouraging prognosis may mistakenly be given. The prognosis is invariably bad. Without any warning, the temperature may shoot up to 104—106°F., remaining at this level for several days and defying all efforts at reduction. The inevitable end is already in sight.

The further progress of the disease resolves itself into a struggle between the latent resistance of the animal and the debilitating effects of the fever, combined with the too plainly obvious anaemia and threatening jaundice. Within a few days, pneumonia may supervene frequently complicated by jaundice, or a sudden fatal bowel or nasal haemorrhage may close the scene. The pneumonia which is so frequently the direct cause of death shows a marked tendency to be gangrenous in type. Many animals may survive for surprisingly long periods, especially when carefully nursed. Of two pedigree fox-terriers affected, one survived for two months. At the end of five weeks it was apparently cured (after treatment). A serious relapse occurred two weeks later, the animal eventually succumbing to debility and severe secondary anaemia. The second animal survived for fifty-three days, showing no mitigation of illness, and dying in the same fashion. Natural recovery has been noted in only a few dogs and in such cases, keratitis is frequent.

#### PATHOLOGY

In all clinical cases of the disease, blood examination has been done as a routine. The majority of such examinations were made upon dogs admitted as fever cases to the Punjab Veterinary College, cases in which, after several negative blood tests, a negative diagnosis of ' tick fever' was given. In most cases, the owner was reluctant to subject the patient to daily blood examination over a protracted period, but in those cases where such facilities existed, especially in the case of the writer's own dogs, the results of the following daily examinations were recorded throughout the course of the illness :—

- (i) Direct examination of Giemsa-stained blood films for the presence of piroplasms or other protozoan parasites;

- (ii) Haemoglobin percentage estimation (Tallquist scale);
- (iii) Variations in blood cytology.

The following data emerged from these simple routine observations:

- (a) At the onset of fever, there is a marked leucocytosis, many of the leucocytes being of an immature type resembling the premyelocyte;
- (b) Platelets are obviously more numerous;
- (c) Erythrocytes are normal in size, although a peculiar vacuolation is frequent (*corps demi-lune*).
- (d) The haemoglobin percentage is normal;
- (e) As the disease progresses, vacuolation of the erythrocytes increases and large mononucleated cells make their appearance. These are typical histiocytes and two forms, one large and possessed of a kidney shaped nucleus and one smaller, with a rounded nucleus, are observed. The cytoplasm of these cells is hyaline and agranular. Gradually these cells increase in number until they are very evident.
- (f) After the lapse of about two weeks, the haemoglobin percentage falls to about sixty, and from this period to the last week or two of the illness, it fluctuates around this figure.
- (g) It is apparent that there is no destruction of erythrocytes or haemoglobinæmia in the circulating blood.
- (h) The types and numbers of leucocytes present show a definite indication of a 'shift to the left'.
- (i) The changes are progressive and towards the termination of the disease there is a sudden and marked fall in the haemoglobin percentage (20—30 per cent), while pathological changes in the erythrocytes become apparent (normoblasts, polychromasia, punctate basophilia, poikilocytosis, Howell-Jolly bodies)—an unfailing indication of rapidly approaching dissolution. The red blood cell count falls to about two million per cmm.

In some few cases showing such a syndrome, cases in which repeated and unavailing search has been made for protozoan parasites in the blood and in which a negative diagnosis of 'tick fever' has been made, the sudden appearance of *P. gibsoni* in very scanty distribution is startling. It is this fact that has led the writer to devote particular study to this disease. In such cases, one may have to search a very large number of fields (50—100) before their presence can be detected, and, when present, they usually occur singly or in pairs. It is noteworthy that the occasional appearance of the parasite in the blood seems to bear no relationship to the severity of the symptoms or the sharp paroxysmal elevations of temperature. In fact it would appear that *P. gibsoni* is certainly absent from the blood during these phases, only making its appearance on alleviation of the fever.

In the majority of cases, *P. gibsoni* has never been detected in the blood throughout the course of the illness, even in the terminal stages when profound anaemic changes are present.

The *post mortem* picture is typical and easily summarised : changes mainly affect the spleen and liver. In acute cases of a short duration of illness, the spleen is markedly enlarged, increased up to ten times the normal weight, dark red in colour or even chocolate, the follicles enlarged and prominent. In one case, the spleen was grossly enlarged, filling the abdominal cavity, intensely haemorrhagic in appearance and several haemorrhagic infarcts were prominent. The spleen in these cases is softer in texture and somewhat diffused. In more protracted cases it is firmer in texture, somewhat 'fibroid' occasionally, with less prominent follicles. The liver is similarly grossly enlarged : in one case, 16·6 per cent of the body weight. (The percentage weight of the liver to the body weight in health is 3 per cent. Sisson.). The organ appears finely cirrhotic, darker or slatey in colour, the surface granular and studded with petechiae and frequently bile-stained.

The blood is thin and watery.

The systemic lymph glands are swollen and haemorrhagic.

The heart muscle usually shows an acute interstitial myocarditis.

The mucous membrane of the gastro-intestinal tract is covered with a sticky mucous deposit. Petechiae are frequently prominent and occasionally discrete areas of ulceration. The duodenum in its first part is deeply stained with bile and occasionally the pylorus.

In acute cases, the red bone marrow is hyperaemic and increased in amount at the expense of the fatty marrow. In more chronic cases, this change is not so evident.

Histopathological changes have been centred on the spleen, liver and bone marrow.

#### SPLEEN

The follicular lymphoidal cells are increased in number and mitosis is evident, especially in peripheral cells where these seem to be undergoing a transformation into larger cells (polyblast, transitional plasma cells, and macrophages) with a clear cytoplasm and irregular nucleus—a type of cell which is prominent in the red pulp. It is possible that these cells are in actuality cells which have originated from the red pulp and are not derived from the peripheral cells of the follicle, which they would then appear to infiltrate. The inconspicuous and shrunken appearance of the follicles in chronic cases would seem to support the idea and it appears probable that in an advancing infection the red pulp of the spleen, actively mitotic as are its component cells, gradually infiltrates and replaces the white pulp.

The most characteristic changes are in the red pulp. There is a very definite hyperplasia of the cells of this tissue, with prominence of the cords of Billroth. Coincident with the marked hyperplastic changes, degenerative

changes are noted. While mitosis and marked proliferation of the cells is present, karyorrhexis is evident in similar cells, the cytoplasm of which stains faintly and appears as a mere rim around the disintegrating nucleus.

The majority of the cells present have clear, round nuclei, in which the chromatin particles radiate from a central mass of nuclear chromatin. These cells present themselves as typical reticular cells and similar cells are found lining the spleen sinusoids. The second type of cell of numerical importance is smaller than the foregoing. The nucleus is compact, deeply basophilic with no internal structure. These are transitional lymphocytes and haemocytoblasts (polyblasts). Interspersed with these are nucleated red blood cells in considerable numbers. The venous sinuses are distended and the lining (littoral) cells of the reticulo-endothelium show distinct mitosis. Many of the hyperplastic (macrophage) cells of the pulp contain blood pigment. A more important observation is that peculiar granules, staining intensely with Heidenhain's iron haematoxylin, are found inside the cytoplasm of the polyblasts and reticular cells. These granules are regular in size and appear in small well-defined clusters. The nucleus of the cells in which they appear is distinct and free from any degeneration. Similar forms, in larger clumps, may be found in extracellular situations, as well as within the cytoplasm of the cells mentioned. These forms stain indeterminately by the Romanowsky stains and judging by a comparison of Heidenhain's stain on sections of the same tissue, it appears that only a few of the bodies can be stained by this method. Pappenheim's stain gives negative results. The changes of importance in the spleen may, therefore, be summed up under three heads, intense and extensive cellular hyperplasia, with the development of polyblasts and macrophages, haemopoiesis, and the appearance of peculiar, structureless inclusion bodies of small size, both within and without the proliferating cells.

#### LIVER

The changes in the liver are associated with an intense congestion. The predominant feature is a reaction of the reticulo-endothelial cells lining the sinusoids and the presence of blood pigment in these cells (cells of Kupffer) which appear larger than normal. Necrosis and fatty degeneration is patchy throughout the lobules and the appearance of an intense cellular infiltration throughout, in some cases, suggests profound toxic excitation of the substance of the organ.

#### THE BONE MARROW

The bone marrow shows changes similar to those described in the red pulp of the spleen, with an erythropoiesis of an exaggerated degree. 'Inclusion bodies' are much more prominent than in the spleen and they are also found in the haemocytoblasts.

## EXPERIMENTAL

A preliminary study of 'Lahore Canine Fever' revealed the essential lesions in the reticulo-endothelial system, notably spleen and bone marrow, with an active erythropoiesis in these tissues. It has been mentioned that, in a small minority of cases, *P. gibsoni* suddenly made its appearance in the peripheral blood, and in a transient fashion, frequently towards the termination of the disease. As such cases were maintained in tick-free dog kennels, it was unjustifiable to assume an intercurrent infection as an explanation of the phenomenon. Moreover, as the symptomatology and haemocytology of these cases which had, perforce, to be acknowledged true *gibsoni* infections ran a course parallel to true 'Lahore Canine Fever' in which no parasites could be determined, and as the essential pathology was similar, a suspicion led to the possibility that 'Lahore Canine Fever' is, in actuality ' tick fever ' due to *P. gibsoni*.

It became necessary, therefore, to study the syndrome and pathology of dogs artificially infected with *P. gibsoni*.

The chief difficulty lay in obtaining suitable experimental animals. The reaction of the adult pariah dogs obtained from Lahore city, when inoculated with *P. gibsoni*, is erratic. In a few cases, the inoculation of blood containing *P. gibsoni* resulted in a *P. canis* infection. The source of the *P. gibsoni* blood used in the transmission experiments was a European owned dog which never, at any time, during observations over seven months, showed any *P. canis* in its blood. As *P. canis* is always easily demonstrable when present, the assumption is valid that this parasite was not introduced accidentally into the experimental dogs along with the *P. gibsoni*. The only explanation that seems to offer a solution is that the experimental dogs were naturally, if latently, infected with *P. canis* and that the introduction of *P. gibsoni* flared up the infection. A considerable number of dogs entirely failed to react to the infection. It must be mentioned that experimental dogs were observed for two weeks prior to inoculation and were maintained in positively tick-free kennels.

It quickly became evident that, if any progress were to be made with the investigation in hand, experiments would require to be conducted on puppies that had been reared in tick-free kennels. It was considered necessary, in view of the racial immunity factor, to use dogs of a pure imported strain, but as this proved an obstacle, the pups used for experiment were from pariah bitches and cross-bred dogs, in one case, a pure brad dog. By this method, it was possible to secure several litters from which the best pups were selected and sixteen puppies were put to experiment.

These were all artificially infected in course of time. The majority were used in experimental treatment but seven remained untreated for study of the natural disease.

Puppies Nos. 3 and 4 were subcutaneously inoculated with 2·5 c.c. virulent blood and failed to react over a period of observation of two months. Puppy No. 13 was inoculated on 25th June 1935 with virulent blood and failed to react. One month later it was inoculated with 5 c.c. spleen emulsion in

normal saline from puppy No. 6, an acute experimental case (Chart No. 1), which had been subjected to experimental treatment with S. U. P. 36. No reaction occurred to the second inoculation but on 11th August 1935, the picture of an early commencing secondary anaemia was obtained in blood film examination. The puppy was then destroyed by electrocution. No parasites had been observed in its peripheral blood. The weight of this puppy was 22·5 lb. and at *post mortem* the spleen weighed six ounces (*i.e.*, 1·66 per cent of the body weight). According to Sisson, the weight of the spleen in a normal sized dog—it is presumed that this is about 20 lb.—is 1½ to 2 oz. Accepting the lower figure 1½ oz. the normal percentage of the spleen to the body weight is, therefore, in the region of 0·47 per cent. The spleen of puppy No. 13 (Chart No. 5) was, therefore, according to these figures, increased in weight by three and a half times the normal.

The liver weighed 1 lb. 12 oz., *i.e.*, 7·6 per cent of the body weight. It is stated that, in health, the liver of the dog weighs approximately 3 per cent of the body weight.

It will be seen then that, in spite of the failure to react to the inoculation of virulent blood, there was a terminal secondary anaemia, co-ordinated with a decided splenic and hepatic enlargement. The important observation is that, in microscopic examination, the cellular reaction of the spleen and liver was similar to that encountered in 'Lahore Canine Fever', although not so pronounced in degree.

Puppy No. 15 (Chart 6) was a somewhat similar case. It was inoculated on 30th July 1935 with 5 c.c. of spleen emulsion in normal saline from Puppy No. 12 (Chart 4), and observations were continued over seventy-one days. The temperature remained practically normal over the first month and no parasites were observed in daily examination, nor were there any pathological alterations in the blood. The temperature over the second month ran only one to two degrees, higher than in the previous month, and on the forty-fifth day was 103·6°F. On the forty-ninth day, decided anaemic changes commenced to show in the blood and these were strongly progressive. *P. gibsoni*, in very scanty numbers, averaging one to two per field, made its appearance on the sixty-fifth day, and was present in equally scanty numbers until the seventy-first day, when the puppy died.

The weight of this dog was 21½ lb. At *post mortem*, the spleen weighed eleven ounces, *i.e.*, the percentage weight of the spleen was 3·2 per cent of the body weight, or more than seven times the normal. The liver weighed 1 lb. 4 oz. or 5·3 per cent of the body weight, *i.e.*, nearly twice the normal weight. The spleen in this case was livid in colour, the pulp was soft in consistence and haemorrhagic infarcts were noted. The liver was fatty and distinct acute interstitial myocarditis was present, with areas of patchy necrosis of the muscular fibres. Histopathological lesions similar to those of 'Lahore Canine Fever' were found in the spleen and bone marrow. The liver was not affected to such a marked degree.

Puppy No. 16 was subcutaneously inoculated on 1st August 1935 with 5 c. c. of a normal saline suspension of spleen and bone marrow from Puppy No. 11, which showed numerous, if periodic, *P. gibsoni* in its blood during life. This case was observed for eighty-six days. On the thirteenth day, the normal incubation period of a *P. gibsoni* infection, there was a mild upward trend to a slightly higher temperature level and in the first half of the second month, the temperature fluctuated between 103—104°F., with *P. gibsoni* absent. Anaemic changes set in about this period and continued progressively until the end, when the haemoglobin percentage had fallen to thirty per cent and a gross haemocytological picture was present. On the seventy-sixth day, until death on the eight-sixth, *P. gibsoni* was evident although somewhat scantily. At *post mortem* the liver and spleen were enlarged (unfortunately these organs were not weighed) and the bone marrow noted as being markedly hyperaemic. Acute interstitial myocarditis was noted. The spleen was markedly diffused. The histopathological changes affecting the spleen, bone marrow and liver were those described in 'Lahore Canine Fever'.

No particular disease syndrome was noted in Puppies Nos. 16, 15, and 13 save towards the end when debility accompanied the profound secondary anaemia.

The case of Puppy No. 10 (Chart 3) was, however, of a different category and here there was a marked syndrome corresponding very closely to that of an authentic 'Lahore Canine Fever'. The temperature reaction was not so pronounced, but the symptomatology was identical. Puppy No. 10 was inoculated subcutaneously with 4 c. c. of citrated blood containing *P. gibsoni* and observations were extended over fifty-two days when death occurred. There was a slight rise of temperature on the twelfth day after inoculation, becoming very definite on the twentieth day. From the twenty-first day to the twenty-fourth, *P. gibsoni* was found in numbers in the blood, and not again during the course of the illness. Secondary anaemia was noted on the thirty-second day with 70 per cent haemoglobin which dropped steadily to 30 per cent four days before death.

The *post mortem* and histopathological examinations were similar to those seen in Puppy No. 16 (Chart 7).

Puppies Nos. 6 and 7 which were selected for experimental treatment, are further examples of cases, which showed febrile reactions with slowly developing and progressive secondary anaemia with erratic and scanty presence of *P. gibsoni* in the blood during these developments. The charts of these two dogs prior to treatment are given to illustrate the point (Charts 1 and 2). Puppy No. 6 was inoculated on 9th February 1934 with 2 c.c. virulent blood. Eighteen days after inoculation, the temperature rose three degrees and slowly abated to normal on the thirty-first day, when, after repeated examinations, only one *P. gibsoni* could be seen in the blood films examined. The haemoglobin percentage had, at this date, fallen to 70. At this stage, secondary anaemia was evident. The parasite did not reappear until the forty-fourth day, when it was very scanty. The following two days it was definitely absent. On the

forty-seventh day it was numerous, after which it disappeared until the eighty-ninth day. On the fiftieth day the haemoglobin percentage was 50. Treatment with S. U. P. 36 was commenced on the fifty-sixth day and thus removed the case from the field of comparative clinical consideration.

Puppy No. 7 (Chart 2) was inoculated subcutaneously with 1 c.c. virulent blood on 28th March 1934 and treatment with tryparsamide was commenced on the 21st May 1934. A febrile reaction commenced early in this case, a typical 'swinging' fever very similar to that seen in a clinical case of 'Lahore Canine Fever', if somewhat less marked. Pathological cells appeared in the blood (histiocytes and bi- and tri-lobed leucocytes) on the twenty-fifth day, when the haemoglobin percentage was 80. *P. gibsoni* was found scantily on the thirty-first day and remained till the thirty-fourth day. It did not reappear till the forty-fourth day and in the interval profound secondary anaemia was evident with a haemoglobin percentage of 40. Parasites remained present, in very small numbers from the forty-fourth day until the commencement of treatment.

Although these experiments were complicated by the presence of a certain quantity of pariah blood (*i.e.*, immune blood) in the experimental puppies, yet it will be seen from them that important facts can be deduced in relation to those known concerning 'Lahore Canine Fever' and these show the close analogy between the two diseases. Clinically, both are characterised by a 'swinging' pyrexia. Periodically there is an abatement of fever, when the temperature may drop to within a normal range, succeeded, usually abruptly, by a sharp upward rise. In the experimental dogs, this upward trend coincided with the appearance of *P. gibsoni* in the circulating blood. (See charts). In Puppy No. 10, it will be seen, however, that the appearance of the piroplasm coincides with the remission of fever. In certain authentic cases of 'Lahore Canine Fever' which had, owing to the sudden appearance of *P. gibsoni*, towards the terminal phase of the disease, to be accepted as originally true *P. gibsoni* infections, a similar feature was noted. A most important observation arising from the clinical examination both of cases of 'Lahore Canine Fever' and artificially induced *P. gibsoni* infection is that, within three to four weeks there is evidence in the circulating blood of commencing derangement of the haemopoietic system, especially the reticulo-endothelium of the spleen. This is evidenced by the appearance of histiocytes; and slightly later, by a type of leucocytosis in which the leucocytes are bi- or tri-lobed (shift to the left). The origin of this phase may be argued from the *post mortem* examinations. It probably depends on an invasion of the leucoblastic tissues on the part of the erythroblastic in the medullary centres, an invasion probably accompanied by some degree of toxic change.

The clinical history of Puppy No. 13 enhances the analogy. This case resembled an authentic case of 'Lahore Canine Fever' in every detail, even to the complete absence of *P. gibsoni* in the circulating blood during the disease syndrome. The case of Puppy No. 15 was only slightly different. The disease syndrome was similar to 'Lahore Canine Fever', but *P. gibsoni* made its

appearance in the last few days of illness. This case corresponds very closely with those cases just mentioned in which, after weeks of negative blood examination, a case is accepted as authentic 'Lahore Canine Fever'—a diagnosis only to be confounded by the sudden appearance of the parasite a day or two before death.

Sufficient has been said, therefore, to prove that from the clinical standpoint, there is a very strong analogy between the two diseases.

It is on *post mortem* and histopathological examination that the suspicion becomes evident. Both diseases possess the following features in common: profound secondary anaemia, marked enlargement of the spleen and liver, hyperaemia of the bone marrow and increase of the red at the expense of the white marrow. The heart muscle lesions common to both diseases have been commented upon. The histopathological features of the spleen, liver and bone marrow have been described in some detail and the only differences between a case of experimental *P. gibsoni* infection and a case of authentic 'Lahore Canine Fever' are differences of degree and not of kind.

The most important observation, however, is that in 'Lahore Canine Fever', no true ring forms of *P. gibsoni* can be seen in sections of the blood-forming tissues, even in Giemsa and Leishman stained preparations. This would appear to vitiate the analogy, but it can be definitely stated that, similarly, no ring forms can be found in the section preparations of *P. gibsoni* infected dogs.

This may be due to a fault in technique but was guarded against by simultaneous staining of sections of the spleen of a bovine which had died from acute *Babesia* infection. The ring forms of this parasite were easily demonstrable by Leishman and Giemsa methods of staining. If, therefore, *P. gibsoni* was present in the tissues examined and was overlooked, the presumption is that it must have been in extraordinarily scanty numbers. If such was the case, it is a striking fact in view of the marked cellular hyperplasia and reticulo-endothelial response, eventuating finally in a reversion to embryonal erythropoiesis of this tissue.

The presence of peculiar inclusion like bodies, both in the littoral cells and the proliferating true reticular cells of the red pulp of the spleen as well as in the cells of the red bone marrow, have been noted in 'Lahore Canine Fever', and equally in artificially produced *P. gibsoni* infection. When the histopathology of 'Lahore Canine Fever' was first studied it was considered probable that these were the products of nuclear degeneration. More extensive study showed that, although these bodies did occur in cells which showed some evidence of degeneration, they were more frequent in perfectly healthy cells in which the nucleus and its membrane were intact. And, furthermore, aggregations of these bodies could be found in extracellular spaces, in some cases in greater numbers than within the cells. It was decided, therefore, to determine, whether these bodies possessed any specificity for the two fevers under study.

A dog was, accordingly, inoculated with a pure strain of *T. evansi* and destroyed at the point of death, after showing a syndrome typical of the infection. The lesions of this disease affect chiefly the haematopoietic tissues, liver, spleen and bone marrow, and are consequent on a rapid destruction of erythrocytes in the circulating blood, with a profound secondary anaemia not clinically unlike that seen in a pure *P. gibsoni* infection. The spleen and liver were markedly enlarged and the bone marrow hyperaemic. Sections of these tissues, stained by Heidenhain failed to show any of the bodies described. Similarly, tissues of the hill bull which had succumbed to a *Babesia* infection were examined with negative results. It appears, therefore, that these bodies are specific for 'Lahore Canine Fever' and artificially produced *P. gibsoni* infection, and as ring forms of *P. gibsoni* are so frequently absent, or at least not found, in such infections, one is led to question whether these bodies do not represent a phase in the development of the life-cycle of *P. gibsoni*. When the infrequency, or complete absence of parasites from the circulating blood is considered in relation to the development of a type of anaemia indicating early involvement of the haemopoietic tissues, it appears a natural assumption that *P. gibsoni* is essentially a parasite of the reticulo-endothelium of the haematopoietic tissues. The appearance, of *P. gibsoni* in the circulating blood would, in these circumstances, be regarded as fortuitous. This is an aspect of the problem, however, which further protozoological study alone can decide. Suffice to say that in Puppy No. 15 which was inoculated with emulsion prepared from spleen of Puppy No. 12 in which ring forms of *P. gibsoni* could not be found and these bodies were numerous, a typical infection resulted, in which *P. gibsoni* was found.

#### DISCUSSION

The literature on 'tick fever' due to *P. gibsoni* is small, little work has been done on the pathology and practically none on the histopathology of the disease. In spite of this, it is difficult to explain the confusion which has existed in the correct interpretation of a disease possessing the syndrome of 'Lahore Canine Fever.' This is a disease showing striking evidence of haematopoietic involvement, similar to that encountered in an authentic case of 'tick fever' and yet there has been, on the part of previous investigators, a reluctance to classify this disease as 'tick fever' due to *P. gibsoni*.

There appear several reasons for this. Sufficient attention has not been paid to the fact that, in true 'tick fever' the presence of *P. gibsoni* in the circulating blood is extremely erratic. It may be found only once or twice during an extended attack of disease, or not at all. In Patton's [1910] original note on the subject, he drew attention to the periodicity of the infection present in the erythrocytes and Symens [1926-27] in the protocols of his excellent publication, clearly indicated the infrequency of parasites in the natural disease.

It appears to have been assumed that, because in the allied infection due to *P. canis*, in which the blood always contains parasites in increasing numbers throughout the course of the disease, the same observation must be true of a *P. gibsoni* infection.

These two infections, though clinically somewhat comparable, run an entirely different course in haemocytological observations, although pathologically comparable in the end result. In a *P. canis* infection there is an early invasion, anisocytosis and destruction of the erythrocytes. The serum is tinged, sometimes deeply, with haemoglobin liberated from the breaking down of erythrocytes and there is an increase of the corpuscular sedimentation rate.

In a *P. gibsoni* infection, there may be an early and transient invasion of the erythrocytes. There is, however, no anisocytosis and destruction of the erythrocytes in the circulating blood, and no evidence of liberated haemoglobin in the serum. (These observations are enhanced by the fact that in a *P. canis* infection, haemoglobinuria is a characteristic symptom, whereas, in a *P. gibsoni* infection, this symptom is never noted.)

At the same time, there is an increased fragility of the erythrocytes in a *P. canis*, which is absent in a *P. gibsoni* infection.

It has already been noted that in an experimentally produced *P. gibsoni* infection, the histiocytes of spleen origin (reticulo-endothelial) early make their appearance. In a true *P. canis* infection, there is no such evidence of an early involvement of the reticulo-endothelial system. Anemic changes occur during the latter phase of the infection and are obviously prompted by the stimulus provided by the erythrocyte destruction and loss. It is believed, from these observations, that *P. gibsoni* is essentially a parasite of the reticulo-endothelium, unlike *P. canis* which appears primarily as a parasite of the erythrocytes.

On this basis, apart from the experimental pathological evidence which has been produced, it is possible to explain the infrequency of *P. gibsoni* in the circulating blood and even its total absence during the course of the disease. And it is then possible to understand the reluctance to accept 'Lahore Canine Fever' as a *P. gibsoni* infection.

In addition to this consideration, previous workers have been impressed by the dysenteric symptoms, accompanied by rapid wasting, which are not infrequent. It is, apparently, these symptoms which have prompted several workers to consider the disease 'Canine Enteric Fever'.

Osmand Bodman and Cooper (unpublished work) noted intestinal ulceration as one of the prominent lesions in their investigations, and it was from such cases that they obtain the *Salmonella* strains mentioned earlier in this note. Symons [1926] and Clive Webb [1906] have drawn attention, however, to the frequency with which dysentery and intestinal ulceration complicate the syndrome of an authentic *P. gibsoni* infection. It is certain that a few dogs normally harbour *Salmonella* strains and it is possible that under the stress of debility induced by the primary protozoan infection, these organisms, potentially latent, assume a pathogenic role. Against this possibility, however, must be mentioned the fact that such cases fail to give a positive Widal test against the *Salmonella* strains isolated.

Lastly, it is believed that much of the refusal to consider the possibility of 'Lahore Canine Fever' as being in reality a *P. gibsoni* infection, has originated in the widespread belief that Novarsenobillon and similar drugs have a specific curative effect in *P. gibsoni* infections. While a very few cases of 'Lahore Canine Fever' have been successfully treated with Tryparsamide and Novarsenobillon, the great majority of cases have failed to respond to treatment. The writer conducted a series of experiments on the treatment of experimentally produced *P. gibsoni* infection in dogs. The results will shortly be published, but at this juncture it may be stated that no drug was found that could be said to have any curative or specific effect in this infection.

In view of the experimental evidence, supported by these observations, it is believed that 'Lahore Canine Fever,' which finds its counterpart in 'Canine Enteric Fever' and 'Canine Typhus Fever' is, in actuality, 'Tick Fever', associated with *P. gibsoni* infection.

#### SUMMARY AND CONCLUSIONS

1. 'Lahore Canine Fever' is one of the most important causes of death of dogs in Lahore and evidence is adduced to show that it is the same disease as has been investigated by workers in other parts of India and designated variously 'Canine Typhus Fever' and 'Canine Enteric Fever'.

2. The disease occurs during the tick seasons, but has not been accepted as 'tick fever', through general inability to demonstrate the causal parasite in the blood of infected dogs, combined with the general refractoriness to treatment with arsenical preparations.

3. The latter aspect of the disease will be dealt with in a later article.

4. An account is given of the clinical features and pathology of 'Lahore Canine Fever' with a comparison of experimentally produced disease of *P. gibsoni* origin.

5. The proof that 'Lahore Canine Fever' is 'tick fever' is based on the following observations:—

- (a) *P. gibsoni* is occasionally, with difficulty, demonstrated in the blood at some stage of the illness, frequently just before death.
- (b) The clinical syndrome of an authentic experimentally-produced *P. gibsoni* infection corresponds in every detail with that of 'Lahore Canine Fever'.
- (c) In both diseases the cytological changes occurring in the blood cells and the method of production of secondary anaemia are parallel.
- (d) The histo-pathological changes in the reticulo-endothelial system, especially in the spleen, reveal important findings which establish the relationship of 'Lahore Canine Fever' to 'tick fever'.

## ACKNOWLEDGMENT

The microphotographs and colour drawings illustrating this article have been prepared by Ahmed Baksh, Assistant Artist of this Institute, to whom thanks are due.

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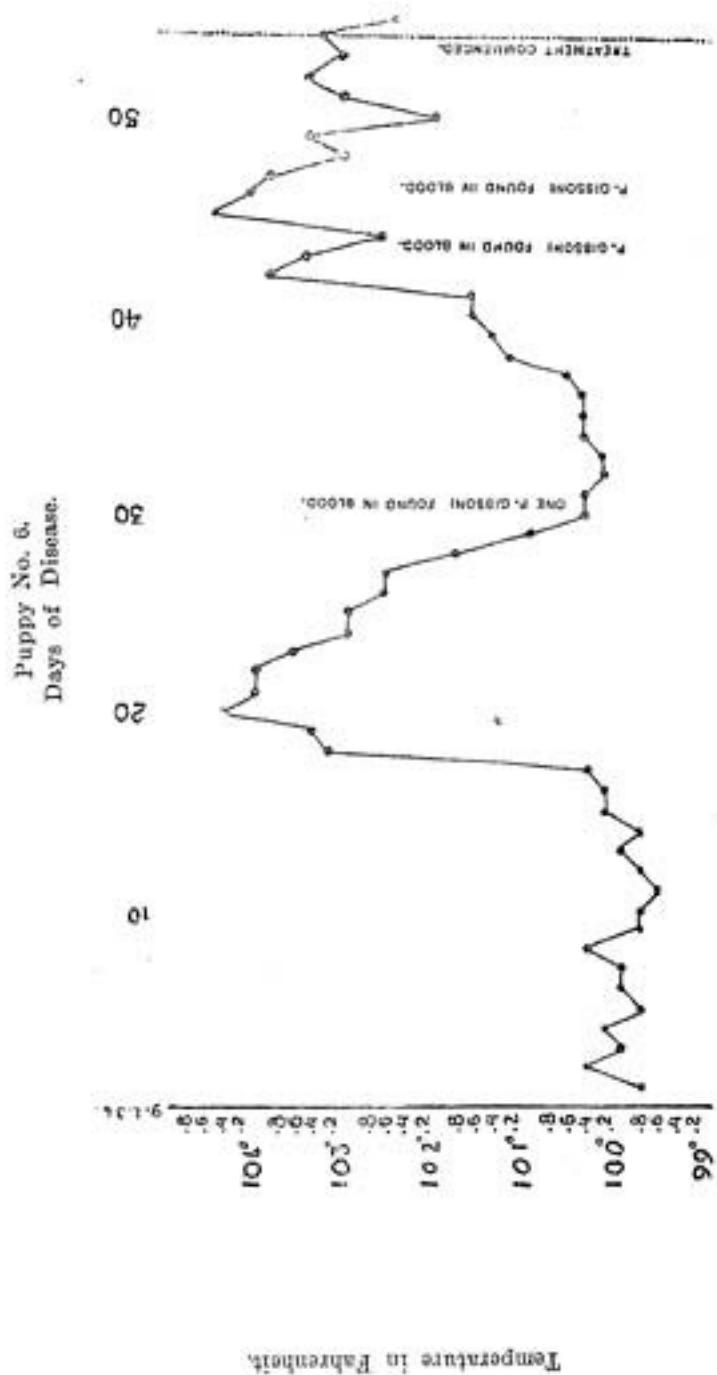
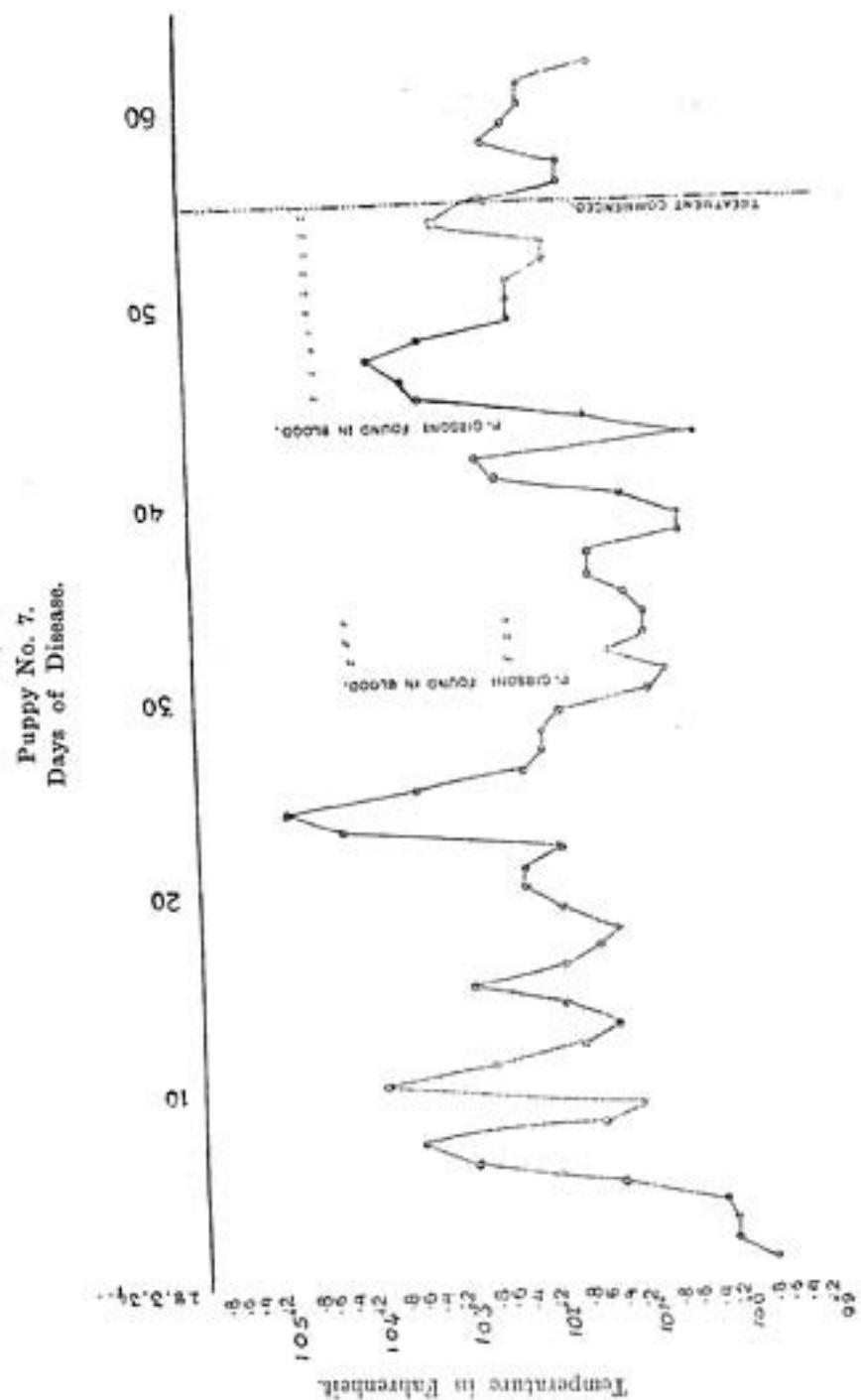


Chart No. 1.



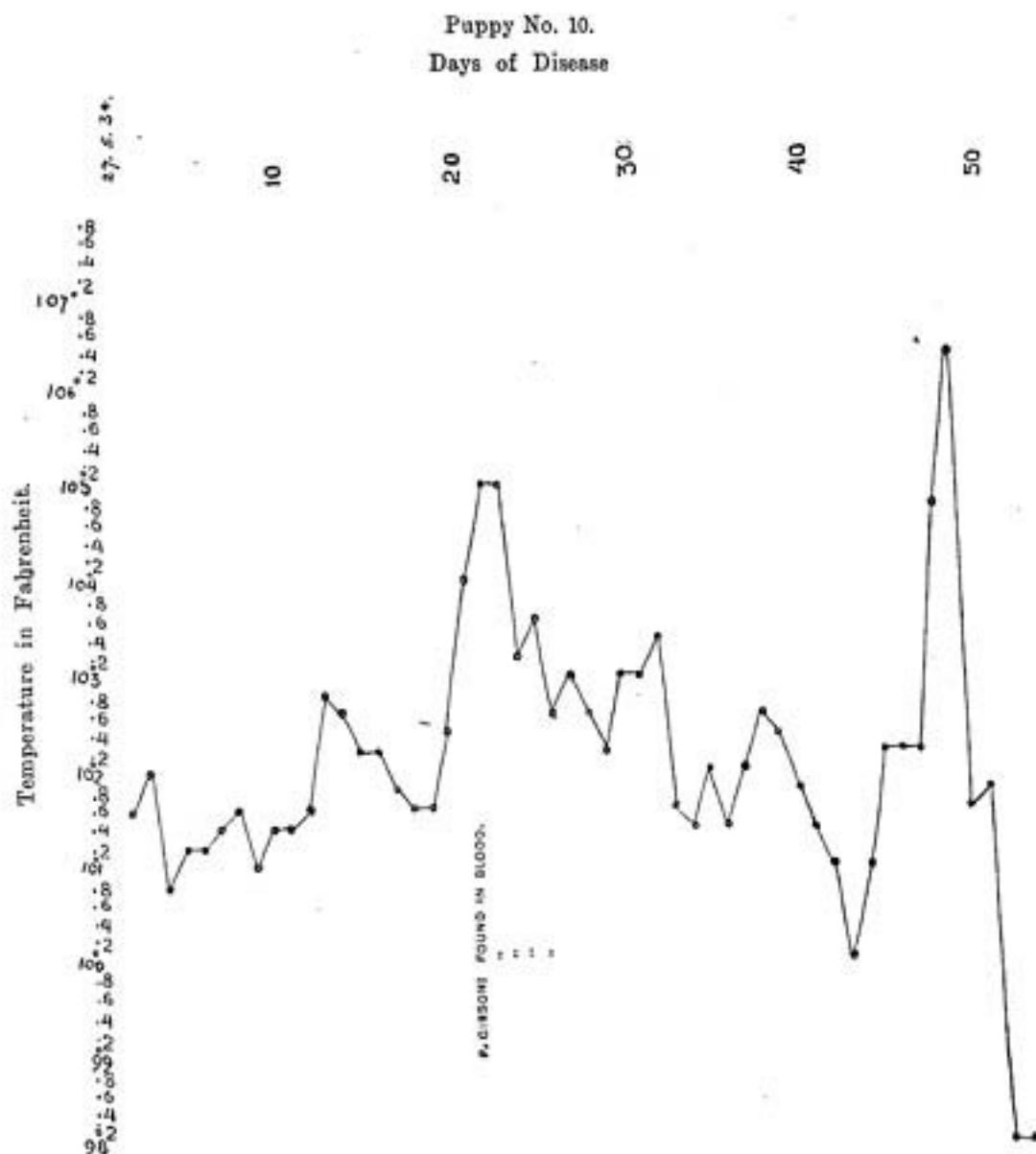


Chart No. 3.

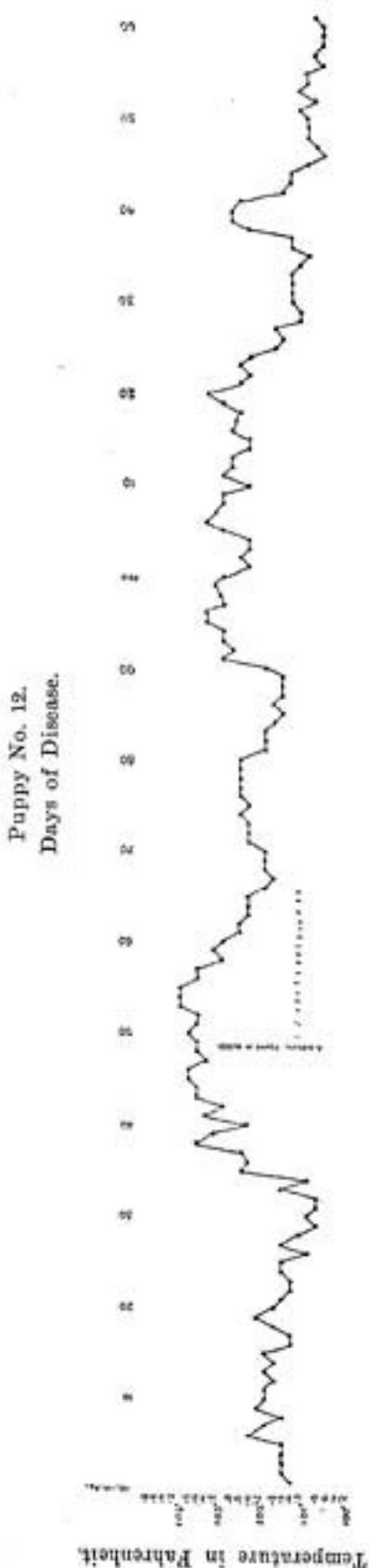
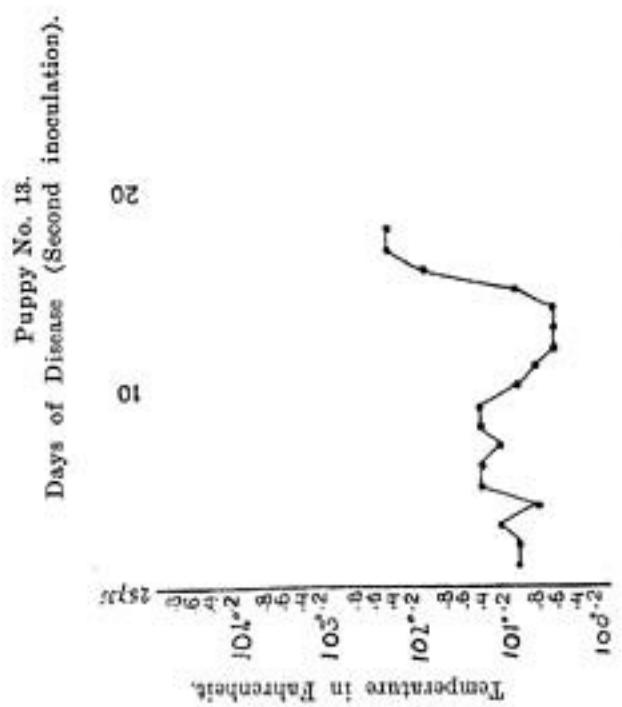


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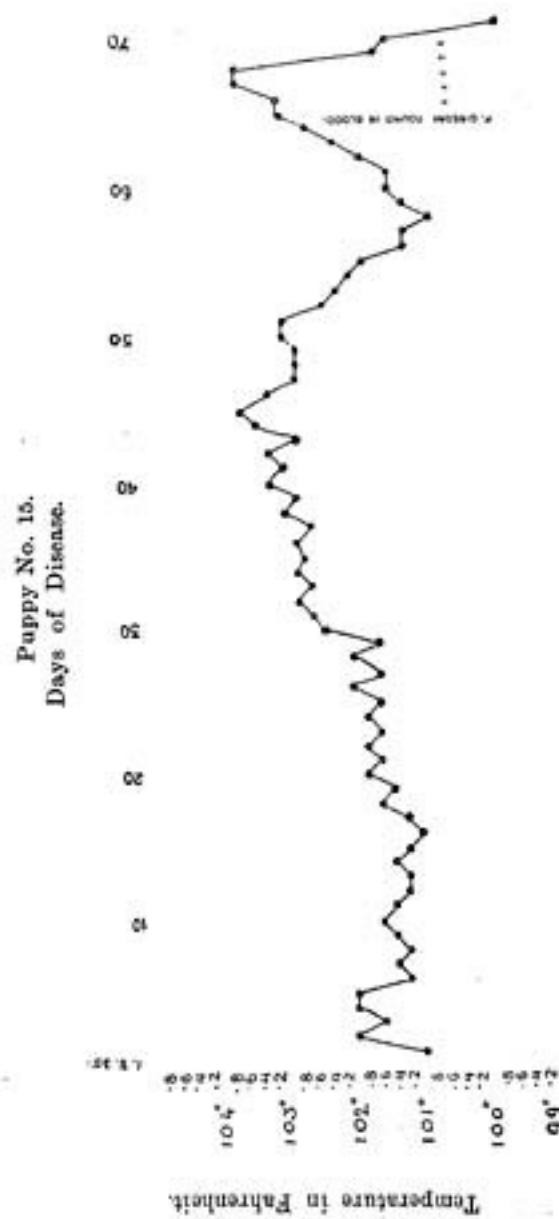


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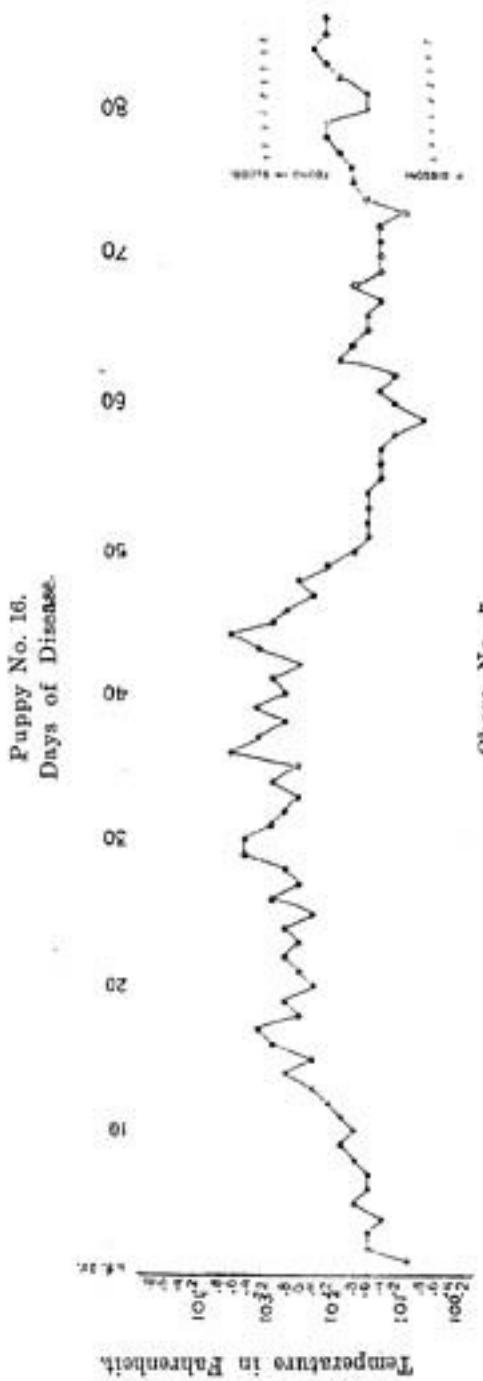
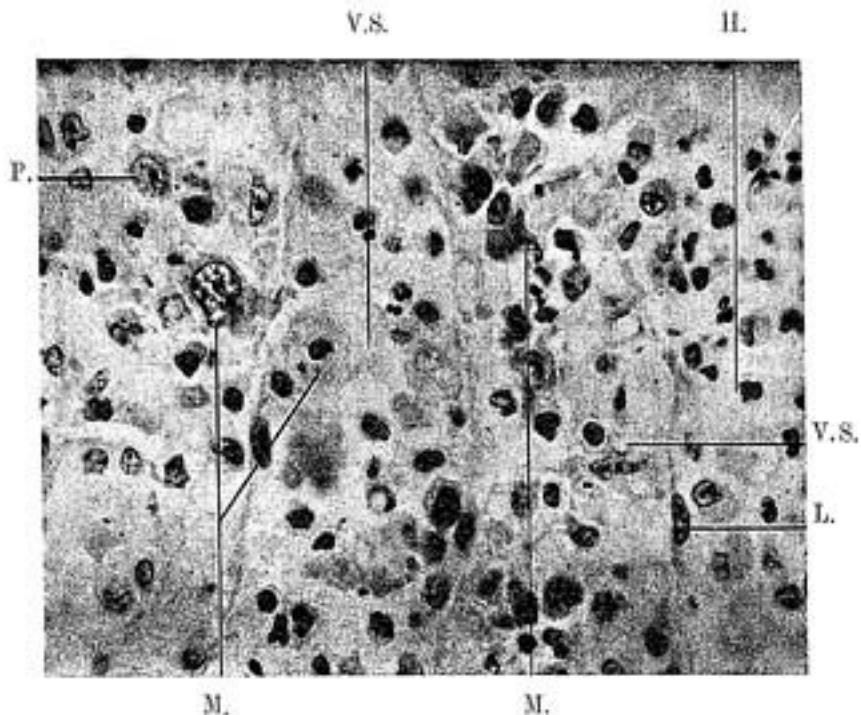


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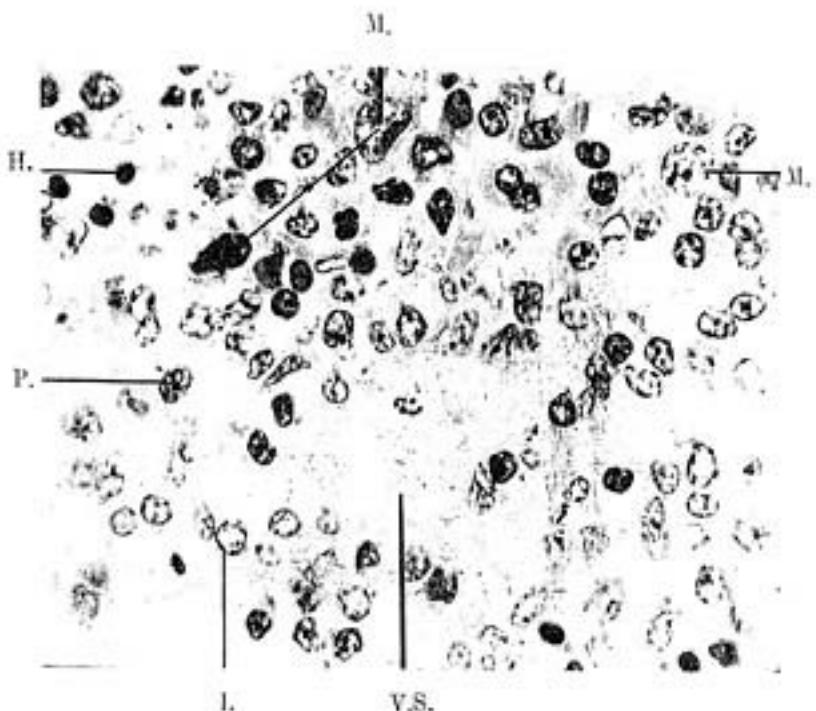




Spleen Lahore Canine Fever  $\times 875$ ,  
Heidenhain's Iron Haematoxylin and eosin.

|      |    |    |    |   |
|------|----|----|----|---|
| V.S. | ++ | ++ | ++ | ... venous sinus                          |
| M.   | ++ | ++ | ++ | ... macrophages in red pulp of spleen     |
| L.   | ++ | ++ | ++ | ... littoral cell of venous sinus         |
| P.   | ++ | ++ | ++ | ... polyblast cell derived from reticulum |
| H.   | ++ | ++ | ++ | ... haemocytoblast                        |

Note the intense polyblastic (reticular cell) hyperplasia with macrophage development. Haemocytoblasts are numerous in the red pulp and venous sinuses

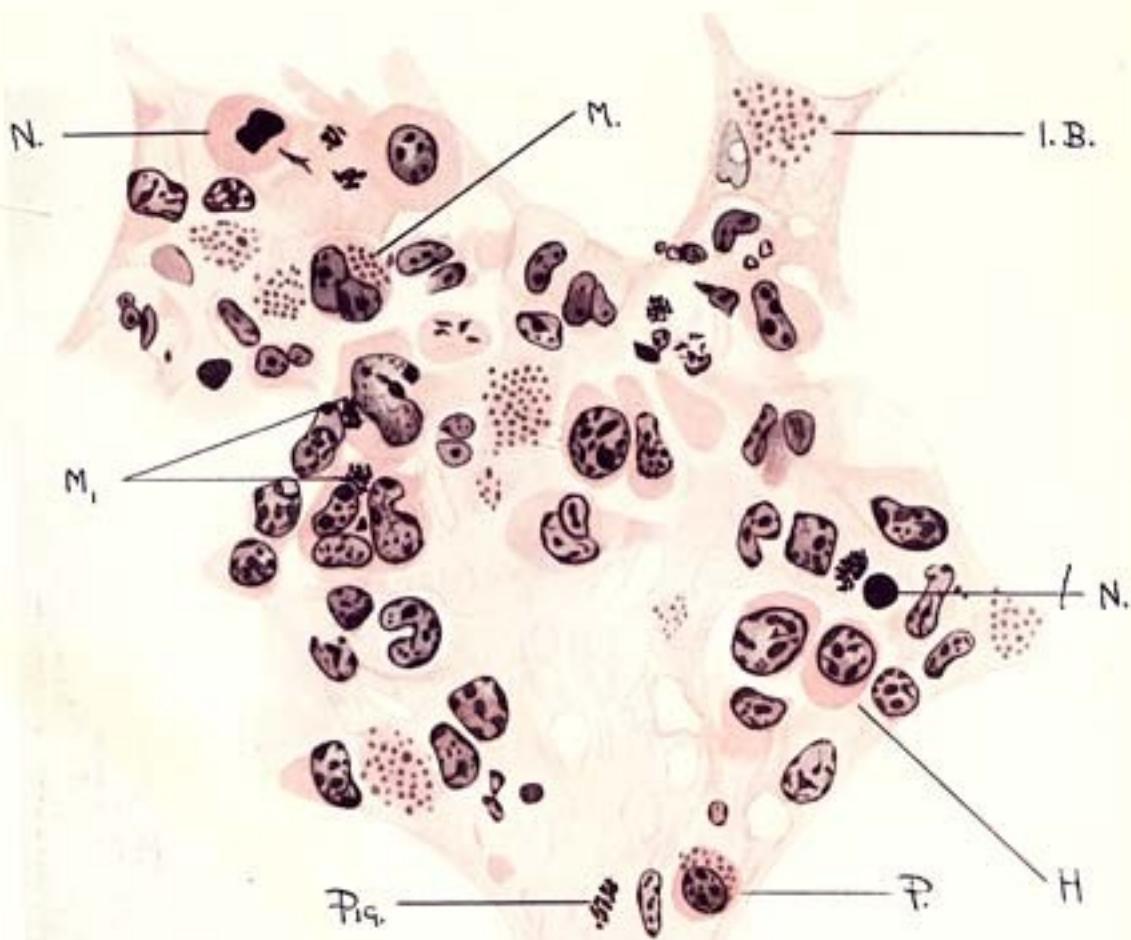


Spleen. Experimental *P. gibsoni* infection.  $\times 875$   
Heidenhain's Iron Haematoxylin and eosin

|      |    |    |    |    |                                       |
|------|----|----|----|----|---------------------------------------|
| V.S. | .. | .. | .. | .. | venous sinus                          |
| M.   | .. | .. | .. | .. | macrophages in red pulp of spleen     |
| L.   | .. | .. | .. | .. | littoral cell of venous sinus         |
| P.   | .. | .. | .. | .. | polyblast cell derived from reticulum |
| H.   | .. | .. | .. | .. | hemocytoblast                         |

Note the cytological changes similar to those in Lahore Canine Fever





Lahore Canine Fever

Section of Bone Marrow, stain Heidenhain Iron Haematoxylin and eosin  $\times 875$

M.—Macrophage with inclusion bodies

M<sub>1</sub>.—Macrophages containing blood pigment

H.—Haemocytoblasts

P.—Polyblast (transitional haemocytoblast) containing inclusion bodies

N.—Normoblasts

I.B.—Inclusion bodies in intercellular spaces

Pig.—Blood pigment



Fig. 1

Experiments A, Japanese infection  
Section of pure culture. Star indicates low transmission rate 60% X 50%

W—Yield per acre  
W—Yield per acre containing 1000 plants  
W—Resistant cell with major pedigree  
W—Resistant cell with minor pedigree  
LW—Infection per cent in intermediate stages  
Z—Zonation  
E—Infestation (translating percentage)  
H—Hatching  
B—Bruit formation

Fig. 2

Japan Chinese Flea.

Infection (resistant) all from pure culture  
The following was sown and harvested and the following infestation rate  
infesting into the intermediate stage fleas. Use the last figure

Fig. 3

Japan Chinese Flea.

Infection (resistant) all from the last crop of the above species containing 1000 plants

FIG. 1

Experimental *P. gibsoni* infection

Section of bone marrow. Stain Heidenhain Iron Haematoxylin and eosin  $\times 875$

- M.—Macrophages
- M<sub>1</sub>—Macrophage containing blood pigment
- R.—Reticular cell with inclusion bodies
- R<sub>1</sub>—Reticular cell with nuclear degeneration
- I.B.—Inclusion bodies in intercellular spaces
- N.—Normoblast
- P.—Polyblast (transitional haemocytoblasts)
- H.—Haemocytoblast
- Pig.—Blood pigment

FIG. 2

'Lahore Canine Fever'

Polyblast (reticular) cell from bone marrow

The cell cytoplasm has swollen and ruptured and the cytoplasmic inclusions are migrating into the intercellular tissue spaces. Note the intact nucleus

FIG. 3

'Lahore Canine Fever.'

Polyblast (reticular) cells from the red pulp of the spleen showing cytoplasmic inclusions

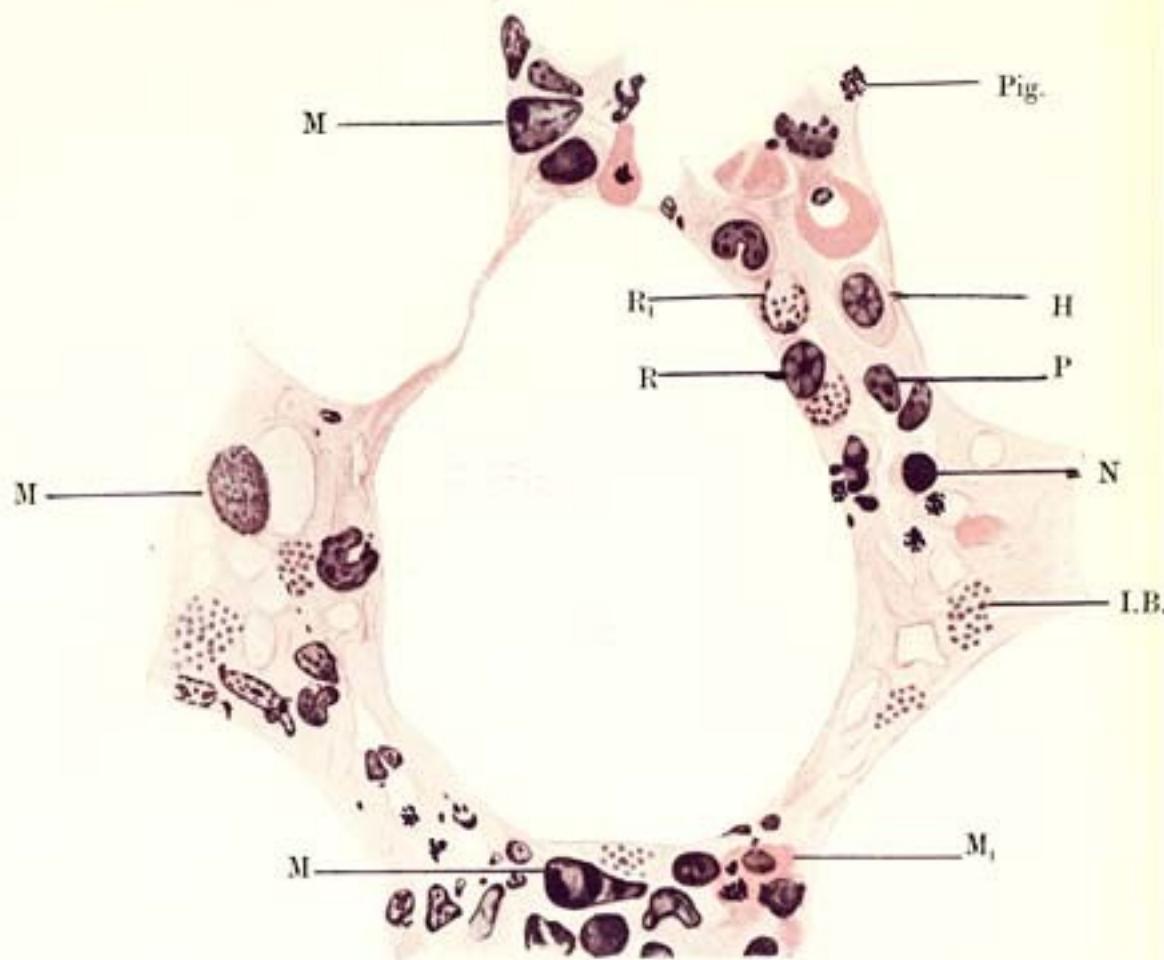


FIG. 1



FIG. 2

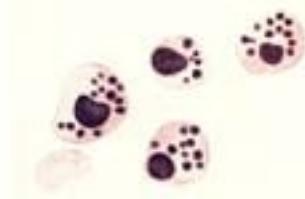


FIG. 3



# STUDIES ON THE GASTEROSTOMATOUS PARASITES OF INDIAN FOOD-FISHES

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(With ten text-figures)

THE earliest work on the Gasterostomatous trematodes is by Rudolph [1819], who described *Monost. crucibulum* and *Monost. galeatum*. The generic names *Bucephalus* and *Gasterostomum* were proposed by Baer [1827] and Siebold [1848] respectively. In 1883, Ziegler pointed out the identity of the two genera and on grounds of priority retained the genus *Bucephalus*. [Braun [1883] created the family Gasterostomidae but as *Gasterostomum* is synonymous with *Bucephalus*, the family name Gasterostomidae lapses into synonymy of Bucephalidae Poche, 1907. The original genus was divided by Diesing [1858] into two genera, *Bucephalus* and *Rhipidocotyle*, a fact which has been overlooked by several subsequent workers. In 1905, Odmer created a new genus *Prosorhynchus* for species with a rhynchus and maintained the genus *Gasterostomum* for forms with an anterior sucker. Nine years later Nicoll [1914] studied the group in detail and divided the family Bucephalidae into two subfamilies, Prosorhynchinae for the genus *Prosorhynchus* and Bucephalinae for the genera *Bucephalus*, *Bucephalopsis* and *Rhipidocotyle*. Ozaki [1924, and 1928] followed Nicoll's classification and added three new genera, *Gotonius*, *Nannoenteron* and *Dolichoenteron*. Issaitachikow [1928] proposed the genus *Skrjabinella* for *Prosorhynchus aculeatus* Odbner, 1905. In 1931, Pigulewsky described a new genus *Mordvilkoria elongata*, and created a new tribe *Gotonia*, but the classification proposed by this author does not appear to be based on sound grounds. The following year Eckmann [1932] published a critical study of the group and, in the light of the anterior adhesive structure of *Rhipidocotyle baculum* which possesses both a sucker and a structure homologous to a rhynchus, pointed out the untenability of the subfamilies Bucephalinae and Prosorhynchinae, which were based on the presence of a sucker or rhynchus. She regarded *Gotonius* and *Nannoenteron* as synonyms of *Prosorhynchus* and *Rhipidocotyle* respectively. Manter [1934] considered *Skrjabinella* as a synonym of *Prosorhynchus*. Yamaguti [1934] maintained the genus *Gotonius* in spite of Eckmann's opinion.

The occurrence of Gasterostomatous trematodes in Indian hosts was recorded by the author in 1934, 1935 and 1937. In this paper are described several new parasites found in Indian food-fishes, both fresh-water and marine.

Order—Gasterostomata Odhner, 1905.

Family—Bucephalidae Poche, 1907.

Genus—*BUCEPHALUS* Baer, 1827.

*BUCEPHALUS INDICUS*, n. sp.

Host—*Macrones seenghala* Day.

Habitat—Intestine.

Locality—Allahabad.

It is a fairly common parasite in the gut of *Macrones seenghala* found in the rivers Ganges and Jumna at Allahabad. The infestation, though never heavy, is nearly fifty per cent during winter months. In the living condition, the parasite is light brown in colour and does not appear to have any marked power of contraction and expansion. The body is elongated and somewhat cylindrical with broadly rounded ends. It is studded with minute backwardly directed spines which are more closely set in the anterior half of body and become sparse towards the hinder end. The parasite in permanent mount measures 1·4—3·2\* in length and 0·28—0·68 in maximum breadth which occurs across the level of the pharynx. The anterior sucker, measuring 0·16—0·36 × 0·14—0·24 in size, has a crown of six well-developed and highly contractile tentacles of 0·095—0·11 × 0·038—0·046 size. The tentacles have a broad triangular base and a little further removed, about 0·03—0·04, from the anterior tip, possess two fairly well-developed lateral processes, 0·03—0·038 × 0·01 in size, situated on either side.

The pharynx is a circular, muscular structure of 0·08—0·14 in diameter situated in the median line at about the junction of the middle and posterior third of body length. The mouth lies in a small depression on the ventral body surface. The pharynx opens posteriorly into a bottle-shaped oesophagus of 0·06—0·18 in length. The intestine is a simple ovoid sac measuring 0·24—0·4 × 0·12—0·2 in size and extending forwards from the pharynx to the anterior level of the ovary.

The two testes are of slightly varying size and shape. They are situated one behind the other on the left side of the body usually in the first two thirds of the posterior third of body length. The anterior testis, measuring 0·14—0·32 × 0·12—0·32 in size, is separated from the posterior testis of 0·12—0·38 × 0·12—0·28 in size by the initial part of the uterus and the vitalline

\* All measurements are in mm.

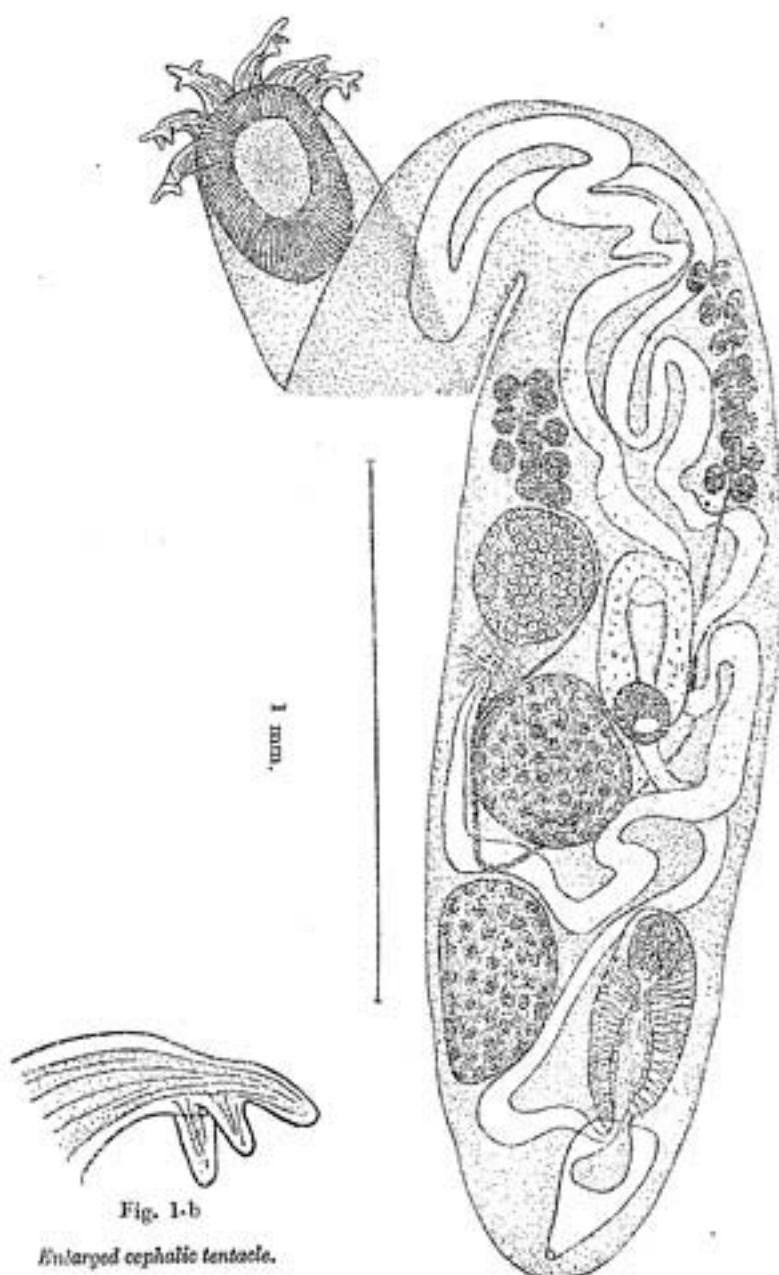


Fig. 1-b

*Enlarged cephalic tentacle.*

Fig. 1-a

*Bucephalus indicus* n. sp.

duct of the right side. The cirrus sac is an elongated, oval structure, measuring  $0\cdot 3 - 0\cdot 5 \times 0\cdot 06 - 0\cdot 2$  in size, and extending anteriorly almost to the level of the anterior limit of the posterior testis. It encloses a small, ovoid vesicula seminalis,  $0\cdot 06 - 0\cdot 14 \times 0\cdot 04 - 0\cdot 12$ , a spindle-shaped pars prostatica of  $0\cdot 16 - 0\cdot 36 \times 0\cdot 04 - 0\cdot 1$  in size, surrounded by prostate glands and a narrow ductus ejaculatorius of  $0\cdot 06 - 0\cdot 14$  in length. The ductus ejaculatorius has a cuticular lining and opens into a roughly triangular genital sinus, at the base of a tongue-like protuberance known as genital tongue, cone or 'Geschlechtszapfen'. The genital sinus is surrounded by numerous gland cells which open into it and secrete the wall of the spermatophore. The genital pore lies subterminally on the ventral surface a little in front of the hinder end.

The spherical ovary of  $0\cdot 1 - 0\cdot 26$  in diameter lies at the junction of the second and posterior thirds of body length close in front of the anterior testis. It is separated from the latter by the compact shell gland complex and the vitelline duct of the left side. The shell gland complex lies between the left body wall, ovary and the anterior testis. A small Laurer's canal is given off from the oviduct just before the latter receives the common vitelline duct. The vitelline glands are composed of small, pear-shaped or oval follicles arranged roughly in pairs along each side of the body. They extend longitudinally from the anterior margin of the ovary to the first third of body length. In contracted specimens the vitelline follicles become aggregated into a mass. The initial part of the uterus runs backwards and passing between the two testes crosses over to the opposite side and continues anteriorly into an irregular coil up to the first sixth of body length and then turns backwards to open into the genital sinus. The whole of the uterus is full of light yellow eggs of  $0\cdot 023 - 0\cdot 027 \times 0\cdot 0114$  in size. The eggs are oval in shape and have a small knob at one end.

The straight, tubular, excretory bladder extends from a little in front of the anterior limits of the vitellaria to the excretory pore which is situated close to the genital opening.

The genus *Bucephalus* was created by Baer in 1827 with *B. polymorphus* as the type species. Though several species have been described under this genus, Eckmann [1932] after a careful study accepts only five species as valid, *B. polymorphus* Baer, 1827, *B. gorgon* Linton, 1905, *B. sp.* Linton, 1910, *B. carangis* MacCallum, 1917, and *B. elegans* Woodhead, 1930. Since the publication of Eckmann's paper five more species have been added—*B. uranoscopi* Yamaguti, 1934, *B. mytili* Cole, 1935, *B. tridentacularia*, *B. aoria* and *B. jagannathai* Verma, 1936. In all, nine species of the genus are known, besides *B. mytili* which is described from larval form.

In its number of tentacles the new species, *B. indicus*, resembles *B. jagannathai*, but it differs from the latter in most of its characters, such as, shape of body, shape and character of tentacles, extent of cirrus sac, vitellaria and uterus and the topography of the gonads, besides differences in measurements. In the nature of its tentacles, topography of gonads, extent of

vitellaria and the shape of excretory bladder, *B. indicus* resembles slightly *B. tridentacularia*, but it differs from the latter in the number of tentacles, anterior extent of cirrus sac and vitellaria, shape of anterior sucker and the difference in the size of the various organs.

*BUCEPHALUS GANGETICUS*, n. sp.

Host—*Macrones scenghala* Day.

Habitat—Intestine.

Locality—Allahabad.

Only two specimens, one immature, of this parasite were collected from the gut of one out of about four dozens of specimens of the host examined. In the living condition the parasite is of light brown colour and exhibits considerable power of contraction and expansion. The elongated and nearly uniformly broad body is covered with minute, backwardly directed spine, and measures  $1 \cdot 76$  in length and  $0 \cdot 38$  in breadth. The anterior sucker has a diameter of  $0 \cdot 2$  and is placed a little subterminally on the ventral body surface. It has a dorsal crown of four cylindrical and highly contractile tentacles of  $0 \cdot 08 \times 0 \cdot 02$  in size. The tentacles are studded with minute, pointed spines.

The small, circular pharynx of  $0 \cdot 06$  in diameter is situated in the median line at the middle of the body length. It leads posteriorly through a very short and narrow oesophagus into a saccular intestine of  $0 \cdot 2 \times 0 \cdot 14$  in size.

The gonads are situated behind one another to the right of the median line in the posterior half of the body. The hinder testis is situated at one sixth of the body length from the posterior end and measures  $0 \cdot 18 \times 0 \cdot 14$  in size. It is separated from the anterior testis, which measures  $0 \cdot 22 \times 0 \cdot 18$  in size, by the initial part of the uterus. The cirrus sac is an elongated, tubular structure measuring  $0 \cdot 74 \times 0 \cdot 14$  in size and reaching anteriorly up to the hinder end of the intestine. It encloses a well-developed, ovoid vesicula seminalis,  $0 \cdot 16 \times 0 \cdot 1$ , a long, tubular,  $0 \cdot 4 \times 0 \cdot 1$ , pars prostatica surrounded by prostate gland cells and a short and narrow ductus ejaculatorius of  $0 \cdot 1$  in length. The ductus ejaculatorius opens into the genital sinus at the base of the genital tongue or cone. The genital sinus is surrounded by gland cells which secrete the wall of the spermatophore. It opens to the outside at the hinder end very close to the excretory pore.

The ovary is pear-shaped and measures  $0 \cdot 16 \times 0 \cdot 1$  in size. It is situated in the space between the anterior third of the anterior testis, posterior half of the intestine and the right body wall. The shell gland complex lies between the two testes and the right body wall. Laurer's canal is present. The vitellaria are composed of small, rounded follicles arranged longitudinally, on the lateral sides of body, beginning from a little in front of the pharynx to the anterior fifth of body length. The uterus is well developed and contains a large number of oval eggs of  $0 \cdot 015-0 \cdot 023 \times 0 \cdot 0076-0 \cdot 0095$  in size. A small, tubular metraterm is present.

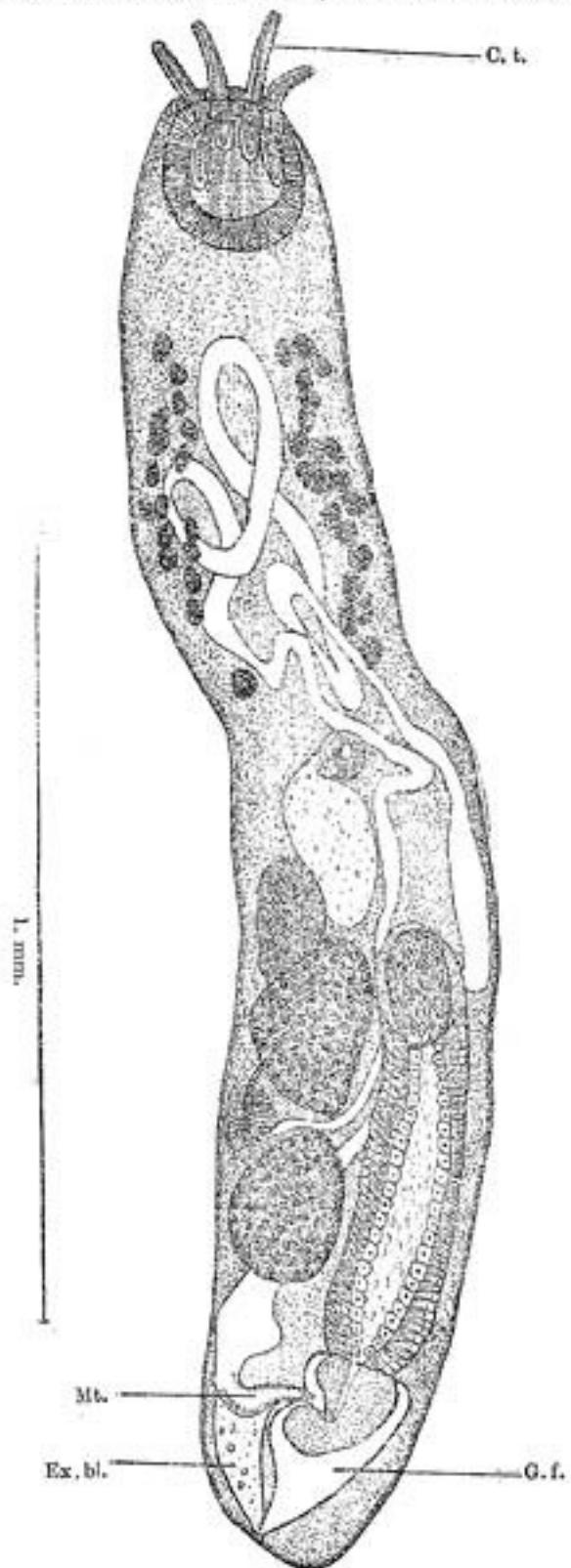


Fig. 2.  
*Bucephalus gangeticus* n. sp.

For 'Key to lettering', please see Pp. 339-340.

The excretory bladder is a long tube extending from the anterior fifth of body length to the hinder end where it opens close to the genital pore.

Linton in 1910 described a trematode as *Gasterostomum* sp. from the gut of *Sphyraena barracuda* which probably has a crown of four tentacles in connection with its anterior sucker. Linton's description of this parasite is extremely meagre. The new species, *B. gangeticus*, resembles the American species only in the number of its tentacles, in case the latter has really four tentacles. However, a careful study of the description and the figure given by Linton shows that the Indian species differs from the American form in most of its characters, such as, shape of body, topography of gonads, position of vitellaria, extent of uterus and cirrus sac, besides differences in measurements.

*BUCEPHALUS BABINA*, n. sp.

Host—*Scatophagus argus* Bloch.

Habitat—Intestine.

Locality—Puri, Bay of Bengal.

Half a dozen specimens of this trematode were obtained from the gut of a marine fish examined at Puri in July 1935. The living parasite is dirty brown in colour and the anterior half of the body is highly mobile. The body is covered with very fine, backwardly directed spines which become smaller and fewer towards the hinder end. In permanent mounts the parasite measures 1.52—2.8 in length and 0.5—0.68 in maximum breadth which occurs across the middle of vitellaria. The anterior sucker measuring 0.14—0.2 × 0.12—0.22 in size is a fairly muscular structure and bears on its antero-dorsal surface a crown of five highly contractile tentacles. They measure 0.076—0.084 × 0.019—0.023 in size and have a number of small, rose-thorn-shaped processes or hooks arranged radially round their basal halves.

The pharynx is a small, transversely oval structure of 0.038—0.068 × 0.06—0.076 in size, situated usually in the median line a little behind the anterior half of the body length. It opens posteriorly into a narrow oesophagus, 0.08 long. The small, sac-like intestine lies in level with the ovary and is of 0.13—0.2 × 0.1 in size.

The two transversely oval testes lie in contact, one behind the other, on the left side close behind the cephalic half of the body. The anterior testis is 0.2—0.32 × 0.16—0.24 in size while the posterior one measures 0.18—0.28 × 0.14—0.18 in size. The cirrus sac lying along the left side is of 0.44—0.7 × 0.12—0.2 in size and extends forwards upto the level of the anterior margin of the posterior testis. It encloses a small, oval vesicula seminalis, 0.12—0.16 × 0.06—0.12, a pars prostatæ, 0.2 × 0.04—0.08, surrounded by well-developed prostate glands and a narrow ductus ejaculatorius, 0.1—0.2 long. The ductus ejaculatorius opens at the base of a well-developed genital tongue extending into the genital sinus. The latter is surrounded by gland cells which secrete the wall of the spermatophore. The genital pore lies sub-terminally on the ventral surface a little in front of the posterior end.

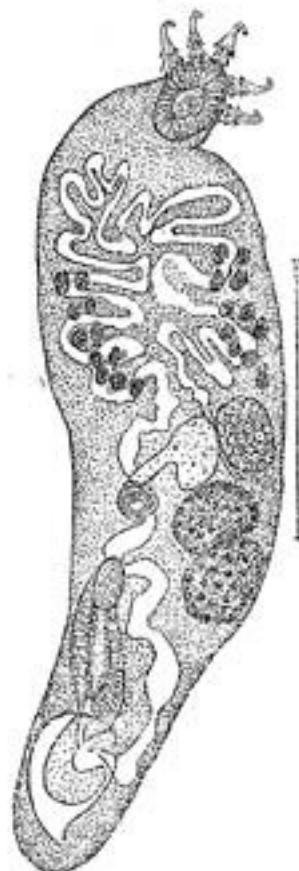


Fig. 3  
*Bucephalus barina* n. sp.

The ovary, measuring  $0\cdot14-0\cdot22 \times 0\cdot12-0\cdot18$  in size, lies in front of the anterior testis at the middle of the body length. It is separated from the anterior testis by a small, compact shell gland mass. A small Laurer's canal is given off from the oviduct. The vitellaria consisting of small rounded follicles arranged roughly in pairs along the lateral sides of body extend from the level of the ovary to the first sixth of the body length. The uterus is well developed and contains a very large number of yellowish-brown, oval eggs of  $0\cdot015-0\cdot019 \times 0\cdot0095-0\cdot011$  in size. Anteriorly the uterus stops a little in front of the suckers.

The excretory bladder is a more or less straight tube extending from the anterior limit of the vitellaria to the hinder end, where it opens to the outside through the excretory pore.

The new species, *B. barina*, differs from the hitherto known species of the genus in the number and character of its tentacles. In its internal anatomy

the parasite resembles *B. jagannathai* rather closely but it can be easily distinguished from the latter by the number and character of its tentacles, besides differences in the measurements.

Key to the Species of the Genus *Bucephalus* Baer, 1827—

|  |  |
|--|--|
| 1. Tentacles four in number . . . . .  | 2  |
| Tentacles more than four in number . . . . .   | 3  |
| 2. Testes one behind the other . . . . .   | <i>B. gangeticus</i> , n. sp.            |
| Testes symmetrically situated . . . . .  | <i>B. sp.</i> Linton, 1910.              |
| 3. Tentacles five in number . . . . .  | <i>B. berina</i> , n. sp.                |
| Tentacles more than five in number . . . . .   | 4  |
| 4. Tentacles six in number . . . . .   | 5  |
| Tentacles more than six in number . . . . .  | 6  |
| 5. Tentacles with two lateral processes . . . . .  | <i>B. indicus</i> , n. sp.               |
| Tentacles with a single inwardly directed process . . . . .  | <i>B. jagannathai</i> Verma, 1936.       |
| 6. Tentacles eight in number . . . . .   | <i>B. tridentacularia</i> , Verma, 1936. |
| Tentacles eighteen in number . . . . .   | <i>B. gorgon</i> , Linton, 1905.         |
| Tentacles seven in number . . . . .  | 7  |
| 7. Tentacles branched at the ends . . . . .  | <i>B. corangis</i> MacCallum, 1917.      |
| Tentacles with lateral branches . . . . .  | 8  |
| 8. Tentacles with one-branch . . . . .   | <i>B. polymorphus</i> Baer, 1827.        |
| Tentacles with two branches . . . . .  | 9  |
| 9. Cirrus sac extends almost to the base of the anterior testis. Tentacles poorly developed . . . . .        | <i>B. elegans</i> Woodhead, 1930.        |
| Cirrus sac does not reach even the hinder margin of posterior testis. Tentacles strongly developed . . . . . | <i>B. uranoscopi</i> Yamaguti, 1934.     |

*B. aoria* Verma, 1936, is not included in the Key as its description is based on badly preserved specimens and its validity is still uncertain.

*B. mytili* Cole, 1935, is described from the larval form only and hence is not included in the Key.

Genus—*BUCEPHALOPSIS* (Diesing 1855) Nicoll, 1914.

*BUCEPHALOPSIS BELONEA*, n. sp.

Host—*Belone strongylura* V Hasselt.

Habitat—Intestine.

Locality—Allahabad.

Four specimens of this trematode were obtained from the intestine of one out of a dozen specimens of the hosts examined at Allahabad. The parasites are inversely pear-shaped with broadly rounded anterior end and a narrow pointed posterior end. The body in permanent mounts measures 1·68–2·5 in length and 0·82–1·2 in maximum breadth across the level of the ovary. It is studded with very minute spines. The large anterior sucker with a diameter of 0·3–0·4 is situated on the ventral surface at the

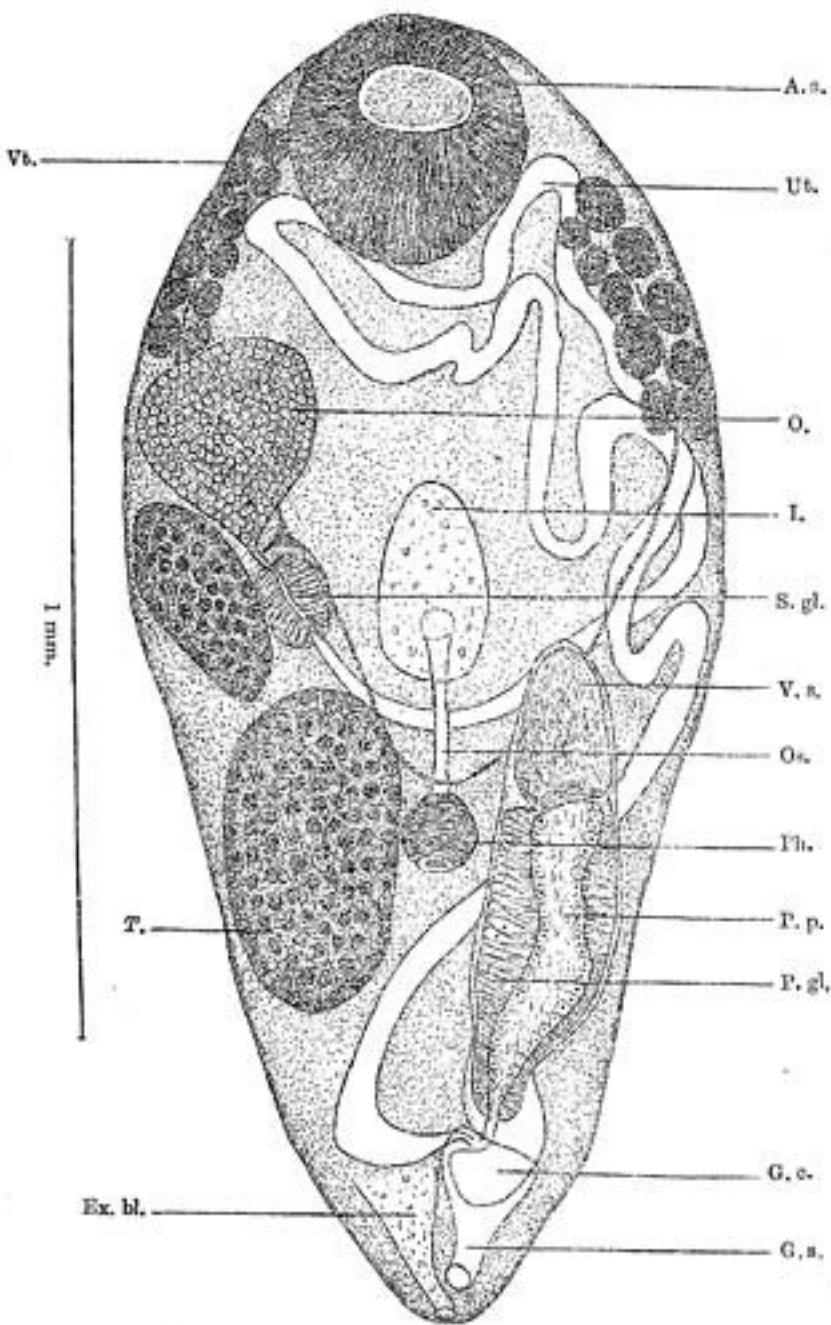


Fig. 4  
*Bucephalopsis belonea* n. sp.

For 'Key to lettering' please see Pp. 339-340.

anterior end of the body. The pharynx surrounding the mouth is a fairly muscular structure measuring  $0.1 - 0.12$  in diameter. It is situated in the median line at the junction of the middle and posterior thirds of the body length. The oesophagus is a straight, narrow tube of  $0.2 - 0.25$  in length. The intestine is sac-shaped and measures  $0.26 - 0.35 \times 0.14 - 0.2$  in size. It is situated in the median line in level with the anterior testis.

The posterior testis,  $0.42 - 0.6 \times 0.24 - 0.3$  in size, lies between the pharynx and the right body wall, extending from the last quarter of the body to the level of the hinder margin of the intestine. The anterior testis of  $0.24 - 0.4 \times 0.14 - 0.26$  in size lies obliquely in front of the posterior testis, between the right body wall, posterior half of ovary and the shell gland complex. The cirrus sac is highly developed and measures  $0.66 - 0.9 \times 0.2 - 0.3$  in size. It extends forwards up to the level of the anterior end of the oesophagus and encloses a vesicula seminalis,  $0.2 - 0.3 \times 0.14 - 0.2$ , a pars prostatica surrounded by gland cells,  $0.36 - 0.54 \times 0.1 - 0.15$ , and a narrow ductus ejaculatorius,  $0.1 - 0.15$  long. The genital tongue extends into the funnel-shaped genital sinus which opens to the outside on the ventral surface a little in front of the hinder end.

The ovary is pear-shaped and lies to the right of the median line extending from the level of the middle of the anterior testis to first quarter of body length. It measures  $0.26 - 0.32 \times 0.26 - 0.3$  in size. The oval, compact shell gland mass lies immediately behind the ovary. A short Laurer's canal is given off from the oviduct. The vitellaria are composed of large rounded follicles arranged longitudinally in pairs on the lateral sides of the body, extending from the level of the middle of ovary to that of the anterior sucker. The uterus extends forward up to the anterior limit of vitellaria. It contains a large number of light brown eggs of  $0.034 - 0.036 \times 0.011 - 0.013$  in size.

The excretory bladder is as in *B. karwei* Bhalerao, 1937.

Diesing in 1855 created the sub-genus *Bucephalopsis* for the larval form of *B. haimeanus* which subsequently proved to be larva of *B. gracilescens* (Rud., 1890). Nicoll [1914] raised the sub-genus to the status of a genus with *B. gracilescens* as the type. A number of species have since been described under the genus, but Eckmann [1932], after a critical study, maintains only eight species—*B. haimeanus* Lacaze-Duthiers, 1854; *B. gracilescens* Rudolphi, 1819; *B. triglae* van Beneden, 1870; *B. arcuata* Linton, 1900; *B. pusilla* Stafford, 1905; *B. exilis* Nicoll, 1915; *B. elongata* and *B. latus* Ozaki, 1928. The description of *B. arcuata* and *B. triglae* being inadequate their validity has been questioned by Bhalerao [1937]. Verma [1936] described five new species of the genus—*B. fusiformis*, *B. garuai*, *B. magnum*, *B. confusus* and *B. minimus* from fresh-water fishes of Allahabad. Of these the last three, in the opinion of Bhalerao [1937], are synonymous with *B. garuai*. The minor differences existing between *B. garuai* and the last three species are ascribed to either difference in age or to individual peculiarities. Recently Bhalerao [1937] has described a new species—*B. karwei*—from the intestine of *Belone cancila* from Poona and has given a key to the valid species of the genus.

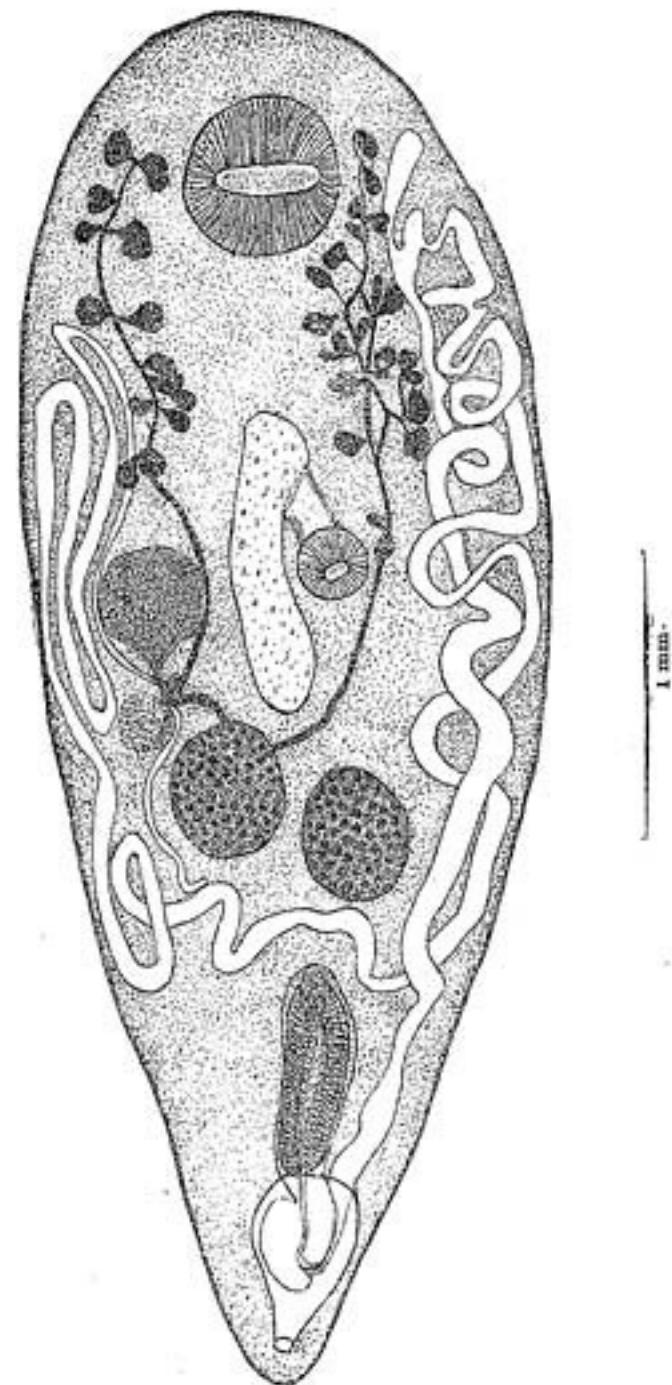


Fig. 5  
*Bucephalopeis garuai*,

In its systematic relationship the new species, *B. belonea*, stands nearest to *B. karwei*. It resembles the latter in the shape of the body and the excretory bladder, in the large size of the anterior sucker, relative positions of gonads, pharynx, excretory and genital pores and the position and the extent of vitellaria. *B. belonea*, however, differs from *B. karwei* in being twice the size of the latter and with most of its internal structures four times or more the size of those of the latter; apart from marked differences in the anterior extent of the cirrus sac which stops at the posterior level of the anterior testis.

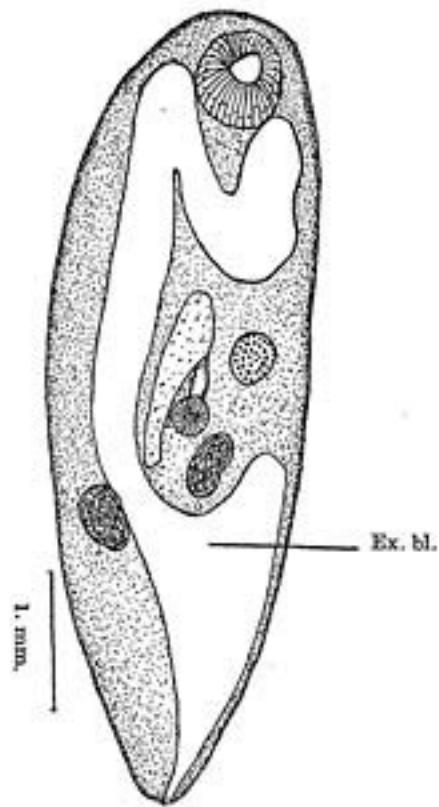


Fig. 6.  
*Bucephalopsis garua*, Excretory system.

|                           | Specimens from<br><i>P. garua</i> . | Specimens from<br><i>S. gangetica</i> . |
|---------------------------|-------------------------------------|---|
| Length of body . . . . .  | 5.73—6.2                            | 3.0—5.96                                |
| Maximum breadth . . . . . | 1.38—1.9                            | 1.26—2.8                                |
| Anterior sucker . . . . . | 0.56—0.69 ×<br>0.59—0.69            | 0.5—0.72 ×<br>0.48—0.72                 |

#### *BUCEPHALOPSIS GARUA* Verma, 1933.

Host—*Silundia gangetica* Cuv. and Val.

Habitat—Intestine.

Locality—Allahabad.

The author submitted a paper on the "Studies on the Gasterostomatous parasites of Indian fresh-water fishes" to the Academy of Sciences, United Provinces, India, on the 14th March 1935 which included the descriptions of *Bucephalus indicus*, *Bucephalus gangeticus* and *Bucephalopsis garuai* Verma, 1936, and *Bucephalopsis fusiformis* Verma, 1936. But as Mr. Verma claimed to have collected his material earlier than the author, the paper was withdrawn.

The species *B. garuai* is represented in the author's collection by a large number of specimens obtained from the gut of *Silundia gangetica* which was found to be nearly always infested with these flukes. Often the infestation was found to be very heavy. The number of specimens collected from a single host varied from 20 to 860. The specimens from *Silundia gangetica* resemble those from *Pseudotropius garua* described by Verma but for slight differences in measurements as given on p. 329.

The author's specimens differ from Verma's in the number of vitelline follicles and the anterior extent of vitellaria, in the shape of excretory bladder and the absence of a muscular sphincter around the genital pore.

*BUCEPHALOPSIS FUSIFORMIS*, Verma, 1936.

Host—*Eutropiichthys vacha* Day.

Habitat—Intestine.

Locality—Allahabad.

The author collected a large number of specimens of this species from the gut of *Eutropiichthys vacha* during the winter months of 1932 to 1935 at Allahabad. The specimens in the author's collection resemble closely the form described by Verma except for the differences in measurements as given below :—

Text-fig. 7, p. 331.

|                            |  | Author's specimens          | Verma's specimens          |
|----------------------------|--|-----------------------------|----------------------------|
| Length . . . . .           |  | 1·26–4·0                    | 1·24–2·52                  |
| Breadth . . . . .          |  | 0·3–0·88                    | 0·39–0·84                  |
| Anterior sucker . . . . .  |  | 0·16–0·26                   | 0·17–0·24                  |
| Pharynx . . . . .          |  | 0·058–0·12                  | 0·07–0·08                  |
| Intestine . . . . .        |  | 0·17–0·34×<br>0·1–0·27      | 0·21–0·46×<br>0·18–0·23    |
| Anterior testis . . . . .  |  | 0·16–0·22                   | 0·18–0·29×<br>0·17–0·26    |
| Posterior testis . . . . . |  | 0·14–0·24                   | 0·16–0·25×<br>0·15–0·21    |
| Cirrus sac . . . . .       |  | 0·46–0·8×<br>0·08–0·14      | 0·46–0·7×<br>0·1–0·14      |
| Ovary . . . . .            |  | 0·12–0·28×<br>0·12–0·18     | 0·14–0·21×<br>0·15–0·17    |
| Eggs . . . . .             |  | 0·019–0·023×<br>0·011–0·015 | 0·013–0·022×<br>0·008–0·02 |

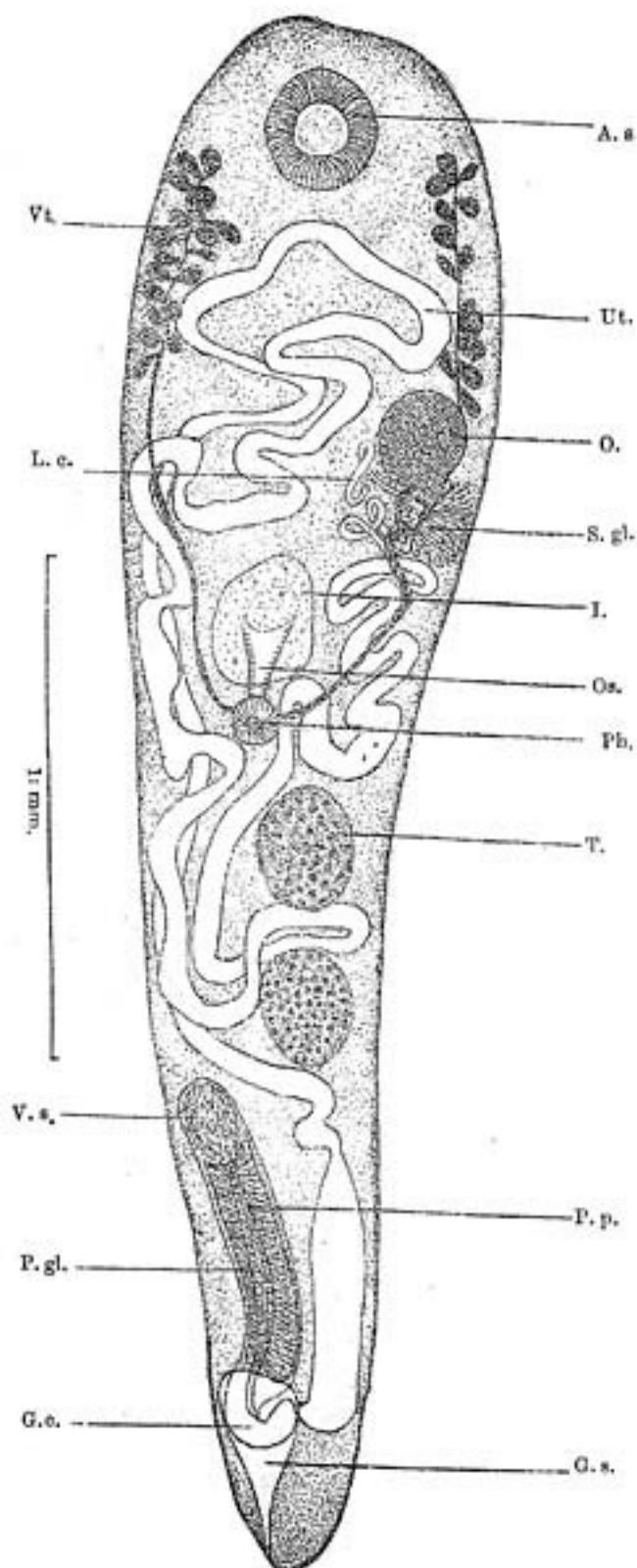


Fig. 7.  
*Bucephaloparis fusiformis.*

For 'Key to lettering' please see Pp. 339-340.

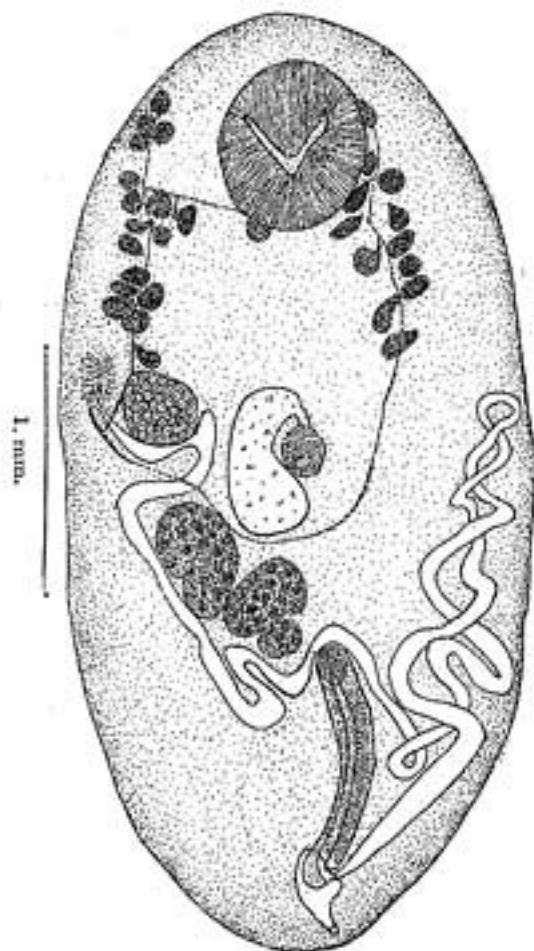


Fig. 8.  
*Buccophalopsis magnum.*

|                            | Author's specimens                                   | Verma's specimens  |
|----------------------------|--|--|
| Length . . . . .           | 3.92   | 8.0-10.0   |
| Breadth . . . . .          | 2.18   | 4.2-4.8  |
| Anterior sucker . . . . .  | 0.66   | 0.3-0.4  |
| Pharynx . . . . .          | 0.24   | 0.46-0.5   |
| Intestine . . . . .        | 0.64 x 0.34  | 1.6 x 0.9  |
| Ovary . . . . .            | 0.32 x 0.3   | 0.38   |
| Anterior testis . . . . .  | 0.5 x 0.34   | 0.9-1.0  |
| Posterior testis . . . . . | 0.4  | 0.75 x 0.84  |
| Cirrus sac . . . . .       | 0.9 x 0.16<br>(About one quarter<br>of body length.) | 1.4-1.6 x 0.3-<br>0.45<br>(Less than one quar-<br>ter of body length.) |
| Eggs . . . . .             | 0.03 x 0.015   | 0.026-0.028 x<br>0.016-0.028   |

*BUCKPHALOPSIS MAGNUM* Verma, 1936.

Host—*Silundia gangetica* Cuv. and Val.

Habitat—Intestine.

Locality—Allahabad.

Only two specimens of this species were obtained from the intestine of one out of more than 150 specimens of the hosts examined at Allahabad. Besides the differences in the measurements of the specimens described by Verma and those collected by the Author, the latter differ from the former in the anterior extent and configuration of vitellaria and in the size and shape of cirrus sac.

Bhalerao [1937] regards *B. magnum*, *B. confusus* and *B. minimus* Verma, 1936, as synonyms of *B. garuai*. From the study of the two specimens which the author has in his collection he is inclined to accept *B. magnum* as a valid species but he maintains that *B. confusus* and *B. minimus* are synonymous with *B. garuai*.

Genus—*PROSORHYNCHUS* Odhner, 1905.*PROSORHYNCHUS MANTERI*, n. sp.\*

Host—*Tetradon oblongus* Bl.

Habitat—Intestine.

Locality—Puri, Bay of Bengal.

This is a very common parasite in the duodenum and intestine of *Tetradon elongatus* Bl. in the Bay of Bengal. A large number of specimens was collected in July 1935. The parasite in the living condition, is capable of considerable expansion and contraction but when fixed under the pressure of a cover-slip, it assumes a fairly regular shape. The body is cylindrical with its anterior end truncated and the posterior rounded. It is covered with small spines which are more closely set anteriorly and become sparse towards the hinder end. In permanent mounts the body measures 0·86–2·26 in length and 0·3–0·64 in maximum breadth. At the anterior end is a prominent, plug-shaped rhynchus measuring 0·1–0·12 × 0·08–0·1 in size. The rhynchus has a thin, muscular wall composed of parenchymatous and glandular cells and fine muscle fibres. The prepharynx is extremely short. Pharynx is a conspicuous structure measuring 0·05–0·1 × 0·05–0·1 in size and situated in the median line at the junction of the first and second quarters of the body length. It leads backwards through a short oesophagus, 0·012–0·06 long, into a bell-shaped gut of 0·12–0·36 × 0·6–0·34 in size. Posteriorly the gut extends up to the anterior margin of the ovary.

The two spherical or oval testes are situated one behind the other, to the left of the median line, in the middle third of body length. The anterior testis, measuring 0·16–0·5 × 0·18–0·56 in size, is separated from the posterior testis by the descending coil of the uterus. The hinder testis, 0·12–0·48 × 0·18–0·5 in size, lies partly overlapping the anterior part of the vesicula seminalis. The cirrus sac is 0·28–0·6 × 0·08–0·14 in size and

\* The new species is named in honour of Dr. H. W. Mander, the well-known American helminthologist.

encloses an oval vesicula seminalis,  $0\cdot05 - 0\cdot1 \times 0\cdot034 - 0\cdot04$ , an elongated, tubular pars prostatica,  $0\cdot15 - 0\cdot3 \times 0\cdot03 - 0\cdot04$ , surrounded by gland cells and a long ductus ejaculatorius,  $0\cdot03 - 0\cdot04$ , which opens at the base of the hook-shaped genital tongue. The genital sinus opens to the outside on the ventral surface a little in front of the hinder end.

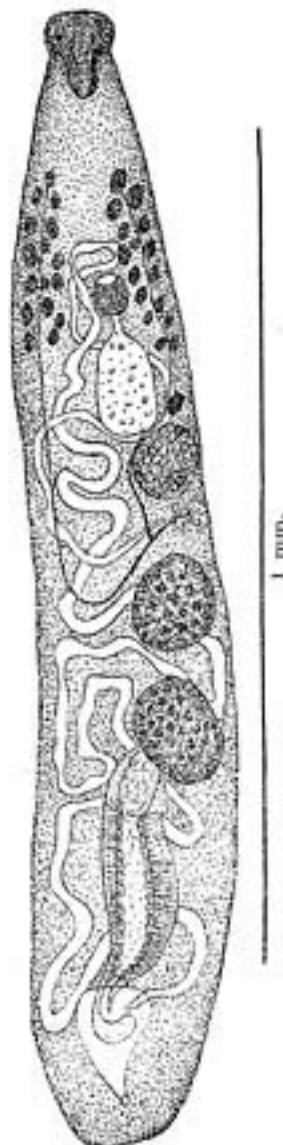


Fig. 9.  
*Protorhynchus manteri* n. sp.

The spherical ovary with a diameter of 0·16–0·44 lies a little in front of the anterior testis, between the compact shell gland complex and the base of the intestine. A small Laurer's canal arises from the oviduct just before it enters the shell gland complex. The vitellaria are composed of small, oval follicles arranged longitudinally in pairs along the sides of the body and extend from in front of the ovary to the anterior one fifth or eighth of body length. The intricately coiled uterus occupies most of the space between the genital sinus and the first quarter of the body length. The eggs are oval in shape and deep brown in colour and measure 0·019–0·02 × 0·011–0·013 in size.

The excretory bladder is a simple, long, tube extending from the anterior level of vitellaria to the hinder end where it opens to the exterior.

Odhner in 1905 created the genus *Prosorhynchus* for the species of Gasterostomes which possess a rhynchus at the anterior end of the body, with *P. crucibulus* Rud., 1819, as the type species. A number of species have subsequently been described under the genus. Ozaki in 1924 described a new species, *P. uniporus*, and a new trematode, *Gotonius facilis*, from the pyloric appendages and intestine of *Inimicus japonicus*. The latter parasite has a cylindrical body and a median ovary, in addition to possessing a rhynchus at the anterior end. Pigulewsky [1931] described a new genus, *Mordvilkovia*, with *M. elongata*, as the type species. Eckmann [1932] dropped the genus *Gotonius* as a synonym of *Prosorhynchus* and definitely included *P. aculeatus* Odhner, 1905, *P. crucibulus* Rudolphi, 1819, *P. facilis* Ozaki, 1924, under *Prosorhynchus*. The account of *P. grandis* Lebour, 1908, was not available to her. Though a rhynchus is known to be present in both *P. triglae* Nicoll, 1914, and *Gasterostomum viperae* van Beneden, 1870, their descriptions are not sufficient to determine their specific characteristics. She considers *P. crucibulum* Manter, 1931, as 'species inquirendae' and doubtfully includes *Gasterostomum* sp. which Linton [1910] described from *Mycloperca venosa*. Tseng Shen in 1930 described *P. vennei* for which Eckmann created the genus *Dollfustrema* in 1934 [Syn. *Dollfusina* Eckmann, 1932]. *Gasterostomum ovatum* Linton, 1910, was raised to the rank of a genus in 1929 by Dollfus who created *Prosorhynchoides* for it. In 1934, Manter described *P. ozakii* and Yamaguti *Gotonius pletycephali*, from American and Japanese fishes respectively. Verma described in 1936 *P. truncatus*, an immature form obtained from a marine fish at Puri.

As a result of a comparative study of the known species of the genera *Prosorhynchus* and *Gotonius*, the author is convinced that the latter is synonymous with the former. In the following table are indicated the shape of the

body and the relative positions of the gonads of the known species of the genus *Prosorhynchus*, from which the identity of the two genera will be clear:—

| Species  | Shape of body    | Position of testes          | Position of ovary  |
|--|------------------|-----------------------------|--------------------|
| <i>P. crucibulus</i> Rud., 1819 . .  | Cylindrical .    | Tandem or obliquely tandem. | Lateral or median. |
| (Syn. <i>G. armatum</i> Molin, 1859,<br><i>Bucephalus crux</i> Levinsen,<br>1881.) |                  |                             |                    |
| <i>P. squamatus</i> Oehlner, 1905) . .   | Oval . . .       | One on either side of body. | Lateral.           |
| <i>P. aculeatus</i> Oehlner, 1905 . .  | Cylindrical .    | Tandem . . .                | Lateral.           |
| <i>G. sp.</i> Linton, 1910 (Fig. 52) . .   | Elongated oval . | Obliquely tandem .          | Do.                |
| <i>P. triglae</i> Nicoll, 1914 . .   | Oval . . .       | Nearly tandem .             | Do.                |
| <i>P. grandis</i> Lebour, 1908 . .   | Oval . . .       | One on either side of body. | Do.                |
| <i>P. uniporus</i> Ozaki, 1924 . .   | Cylindrical .    | Tandem . . .                | Do.                |
| <i>P. facilis</i> Ozaki, 1924 . .  | Elongated .      | One on either side of body. | Do.                |
| <i>P. Osakii</i> Manter, 1934 . .  | Cylindrical .    | Tandem . . .                | Do.                |
| <i>P. platycephali</i> Yamaguti, 1934 .  | Cylindrical .    | Tandem . . .                | Do.                |
| <i>P. truncatum</i> Verma, 1936 . .  | Cylindrical .    | Tandem . . .                | Do.                |

In the shape of its body and the tandem position of its testes the new species, *P. manteri*, resembles *P. crucibulus*, *Gasterostomum* sp. [Linton, 1910, figure 52], *P. facilis*, *P. platycephali* and *P. truncatus*, but it can be readily distinguished from the above five species by its internal organisation. It differs from *P. crucibulus* in the more cephalad position of its pharynx and intestine, the disposition of vitellaria, the anterior extent of the cirrus sac which is overlapped partially by the posterior testis, the position of gonads in relation to the intestine and the absence of filaments in the eggs. From *Gasterostomum* sp. Linton, 1910, it can be distinguished by the position of pharynx, intestine, vitellaria and genital pore and the extent of uterus and cirrus sac. In the position of pharynx and intestine, disposition of vitellaria and the anterior extent of uterus and cirrus sac, it can be easily distinguished from *P. facilis* and *P. platycephali*. The new species differs from *P. truncatum* in most of its characters such as the topography of the gonads, position of the pharynx, intestine, genital pore and vitellaria.

#### *PROSORHYNCHUS ARABIANA*, n. sp.

Host—*Synaptura orientalis* Bloch.

Habitat—Intestine.

Locality—Karachi, Arabian Sea.

Only four specimens, all mature, of this species were recovered from the intestine of its host examined at Karachi in June 1936. The elongated, cylindrical body in permanent mounts measures 3·3–4·5 in length and 0·4–0·6 in maximum breadth which occurs at the level of ovary. The cuticle has extremely fine spines which are hardly visible in *toto* mounts. At the anterior end of the body is a balloon-shaped rhynchus of 0·22–0·3 × 0·16 in size. The small, globular pharynx of 0·06–0·08 in diameter is situated to the left of the

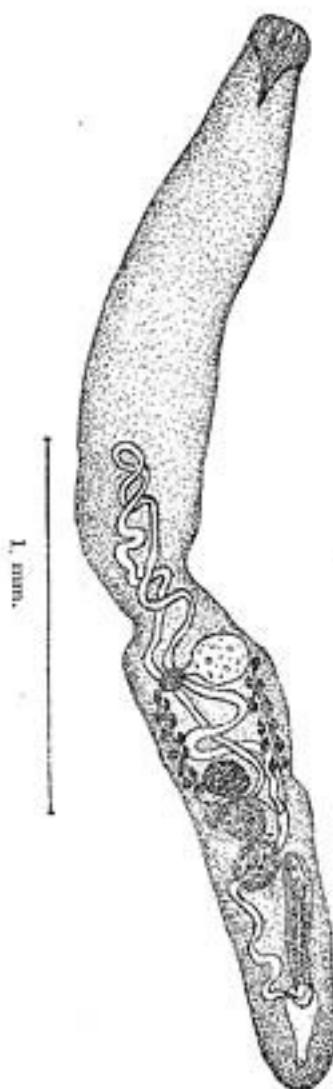


Fig. 10.  
*Troporhynchus arabicus* n. sp.

median line at about three-fifths of body length from the anterior end. It is followed by a small oesophagus which opens into the bulb-shaped intestine,  $0.16 - 0.24 \times 0.12 - 0.15$  in size.

The testes are in tandem. The posterior testis of  $0.1 - 0.12 \times 0.08 - 0.12$  in size lies in the median line in front of the hinder fifth of the body length. The anterior testis measures  $0.1 - 0.12$  in diameter and lies close in front of the posterior one. The small, narrow, club-shaped cirrus sac of  $0.4 - 0.26 \times 0.06 - 0.08$  in size lies along the right body wall, extending forward upto the middle of the hinder testis. It encloses a small vesicula seminalis,  $0.08 - 0.12 \times 0.04 - 0.06$ , an elongated, narrow pars prostatica,  $0.14 - 0.22 \times 0.04 - 0.06$ , surrounded by prostate glands, and a minute ductus ejaculatorius. The small genital tongue is hook-shaped. The genital sinus opens a little in front of the hinder end on the ventral surface of the body.

The small, ovoid ovary  $0.08 - 0.12$  in diameter, lies immediately in front of the anterior testis. The shell gland complex lies in the space between the ovary, anterior testis and the left body wall. A small Laurer's canal is present. The vitellaria consisting of small, pear-shaped follicles and arranged longitudinally in pairs on either side of body extend from the level of the posterior margin of ovary to the level of the pharynx. The uterus contains a fairly large number of eggs and extends from the anterior two-fifths of the body length to the genital tongue. The eggs measure  $0.023 \times 0.012$  in size.

The excretory bladder is a small, elongated, saccular tube extending from the level of pharynx to the excretory pore which is situated near the genital opening.

In having a cylindrical body and the testes arranged in tandem *P. arabiana* resembles the species with which the preceding species has been compared. However, it differs from all of them in the position of its pharynx, intestine, gonads and of the vitellaria, and in the extent of uterus and the characteristic shape of the rynchos, besides differences in measurements.

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#### Key to lettering

|                         |                         |
|-------------------------|-------------------------|
| A. s. . . . . . . . .   | Anterior sucker.        |
| C. t. . . . . . . . .   | Cephalic tentacles.     |
| Ex. bl. . . . . . . . . | Excretory bladder.      |
| G. c. . . . . . . . .   | Genital cone or tongue. |
| G. f. . . . . . . . .   | Genital funnel.         |
| G. s. . . . . . . . .   | Genital sinus.          |
| I. . . . . . . . .      | Intestine.              |
| L. c. . . . . . . . .   | Laurer's canal.         |
| Mt. . . . . . . . .     | Metratarm.              |
| O. . . . . . . . .      | Ovary.                  |

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|        |   |   |   |   |   |   |   |                      |
|--------|---|---|---|---|---|---|---|----------------------|
| Os.    | . | . | . | . | . | . | . | Oesophagus.          |
| P. p.  | . | . | . | . | . | . | . | Pars prostatica.     |
| P. gl. | . | . | . | . | . | . | . | Prostate glands.     |
| Ph.    | . | . | . | . | . | . | . | Pharynx.             |
| S. gl. | . | . | . | . | . | . | . | Shell gland complex. |
| T.     | . | . | . | . | . | . | . | Testis.              |
| Ut.    | . | . | . | . | . | . | . | Uterus.              |
| V. s.  | . | . | . | . | . | . | . | Vesicula seminalis.  |
| Vt.    | . | . | . | . | . | . | . | Vitellaria.          |

AN OUTBREAK OF EQUINE ENCEPHALOMYELITIS  
IN  
A MOUNTED MILITARY POLICE TROOP IN BIHAR

A PRELIMINARY REPORT

BY

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INTRODUCTION

THERE occurs in many parts of India and certain neighbouring countries a disease of horses known as Kumri which is characterized by incoordination of movement and loss of control over the hind limbs. The disease has been under investigation for a very long time but its etiology still remains obscure.

There also occurs on the continent of Europe and in America a group of diseases of virus origin characterized by symptoms of paraplegia and cerebral involvement. Of these, the best known is Borna disease which has existed in Germany for over a hundred years. An analogous disease occurs in France which has been described by Moussu and Marchand [1924].

The disease in America referred to as equine encephalomyelitis was first described by Meyer and his associates [1930] in California and they showed that it was due to a virus, of which there are two immunologically distinct strains, the original western strain of Meyer which is supposed to be identical with the strain causing equine encephalomyelitis in Argentine, and an eastern strain first described by Ten Broeck and Merrill [1933], the latter being the more virulent. In addition there is a Russian strain responsible for the disease in horses in Russia.

Several other types of equine encephalomyelitis have also been described mostly from the continent of Europe, but it is extremely doubtful if there is any relationship between them and whether they are all of virus origin.

In this country, cases of disease in horses, characterized by symptoms of paraplegia, have in the past been either diagnosed as Kumri or attributed to a variety of unproved etiological factors, while no evidence was available until quite recently that the specific form of equine encephalomyelitis such as occurs in America and elsewhere also occurred in this country.

The first outbreak of encephalomyelitis in which a virus was suspected to be the cause occurred in a cavalry regiment at Multan in the winter of 1933. The disease was investigated by Mosley, Heane and Shirlaw [1934], the last-named of whom adduced evidence to show that it was very closely related to the American type of equine encephalomyelitis and that it was probably caused by a neurotropic filterable virus of organotropic type.

Recently a similar outbreak has been described from Kashmir by Kak [1937] and the close clinical similarity between this outbreak and the one at Multan suggests that probably it too was of virus origin.

Within recent years several outbreaks of equine encephalomyelitis have been reported, mostly from military establishments in the Punjab, and frequent references to it are to be found in the recent reports of the Imperial Veterinary Research Institute at Mukteswar and of the Army Veterinary Service in India, but there is nothing to show that the existence of a virus in these outbreaks was proved.

In this article an account is given of an outbreak of paraplegia, later diagnosed as encephalomyelitis, that occurred among the Mounted Military Police horses at Jamshedpur during the winter of 1936-37 and which bore some resemblance to the outbreaks at Multan and in Kashmir but in which the existence of a virus could not be definitely proved. An attempt to connect the outbreak with vegetable poisoning also proved abortive, while examination of the blood and fodder for their calcium and phosphorus contents did not reveal any abnormality of such an exceptional nature as to justify any definite conclusions. As a matter of fact, the etiology of the disease has up to now remained undetermined.

In this connection it is important to note that a similar outbreak has again occurred among these horses this year (1937-38), the first case occurring towards the end of October and the last towards the beginning of January. Of the total number of six horses attacked, four recovered and two are going to be destroyed. Similar cases of a sporadic nature have also been reported from among the Mounted Military Police horses at Patna and some privately-owned horses in North Bihar. These last-named are also Walers except for two which are country-breds.

The description which follows relates only to the outbreak which occurred during the winter of 1936-37 except where otherwise specifically mentioned.

#### HISTORY OF THE OUTBREAK

There are five troops of the Mounted Military Police horses in Bihar, of which three are stationed at Arrah, one at Patna and one at Jamshedpur. Each troop comprises twenty-eight horses and the troops exchange places once every year. The horses are all of the Waler breed. The Mounted Military Police station at Jamshedpur was first established in May 1933 and the troop in which this particular outbreak occurred had been there since October, 1935.

The first case with any symptoms of paraplegia or loss of control over the hind limbs among the Mounted Military Police horses at Jamshedpur occurred in August, 1935. This animal while out on exercise one day went suddenly lame, the symptoms being so acute that it could be walked back to the lines with the greatest difficulty. Later, the acute pain passed off and symptoms of paraplegia were manifested which became progressively worse. Since it was an isolated case and the symptoms shown bore some resemblance to those seen in Kumri, a diagnosis of the latter disease was given and the animal destroyed.

In the meantime the troop was changed and no further cases occurred until the present outbreak started in August, 1936, exactly one year after the case in 1935. The first horse to be attacked during this outbreak was No. 119 which one day about the middle of August was seen to be going definitely unsound in the hind quarters. During September, 1936, another horse No. 31 developed the symptoms as evidenced by the dragging of the off-hind toe at the trot. This case was variously diagnosed by officers on the spot as string-halt or Kumri. Later in the month of October cases arose with greater frequency, as many as five horses being attacked. These were Nos. 2, 52, 8, 70 and 12. During this month the private pony of the Commandant of the troop which had been in contact with the Mounted Military Police horses, while away at Ranchi for races, also developed the disease. On the 29th October, it was noticed to be off colour and refused to lie down. The following day the condition became worse and definite signs of paraplegia became visible. The animal was brought back to Jamshedpur where it became progressively worse and had to be destroyed three months later. During the month of November horse No. 29 was attacked and during December No. 13, after which for three months no further cases occurred, when suddenly in March, 1937, another horse No. 39 developed the disease. This was the last case to occur and though the healthy and affected horses continued to remain in close contact due to lack of facilities for proper segregation, there was no further spread of the disease.

In this connection it may be stated that two horses, belonging to an officer at Patna, which had also been to Ranchi and had come in contact with the Commandant's horse developed, on arrival at Patna, symptoms very similar to those exhibited by the Mounted Military Police horses at Jamshedpur, while isolated cases of the disease also occurred in the troops at Patna and Arrah which had never been in contact with any of the other affected horses.

Of the eleven Mounted Military Police horses attacked at Jamshedpur including the Commandant's, two recovered (Nos. 8 and 13), two died after periods of illness lasting for a month and a fortnight respectively (Nos. 52 and 12), while seven had to be destroyed after they had been kept under observation and treatment for several months (Nos. 31, 2, 70, 119, 29, 39 and the Commandant's).

It will thus be seen that of a total number of twenty-nine horses in the troop (including the Commandant's) eleven developed signs of paraplegia, of which two recovered and the rest died or were destroyed; the incidence and mortality rate thus working out as 38 and 82 per cent respectively.

The following statement giving the numbers of horses and the dates on which they were attacked will show the incidence of the disease in this troop :—

| No. of horse           | Date on which symptoms were first seen |
|------------------------|--|
| 119 . . . . .          | 14th August, 1935.                     |
| 31 . . . . .           | 17th September, 1935.                  |
| 2 + + + + .            | 2nd October, 1936.                     |
| 52 . . . . .           | 14th October, 1936.                    |
| 8 . . . . .            | 22nd October, 1936.                    |
| 70 . . . . .           | 24th October, 1936.                    |
| 12 . . . . .           | 29th October, 1936.                    |
| Commandant's . . . . . | 29th October, 1936.                    |
| 29 . . . . .           | 12th November, 1936.                   |
| 13 . . . . .           | 15th December, 1936.                   |
| 39 . . . . .           | 12th March, 1937.                      |

In this connection it may be stated that Jamshedpur formerly used to be rather an important centre for horse racing in Bihar and a number of horses used to come to Jamshedpur from Calcutta and other places for this purpose. Enquiries made on the spot have shown that losses among these horses from the disease under report or one closely related to it used to be frequent and severe, in many cases the animals getting attacked and succumbing within a short time of their arrival at Jamshedpur. These horses used to be stabled in the vicinity of a certain farm and the popular belief was that they were poisoned by the rass obtained from this farm. The losses were so severe that Jamshedpur came to be looked upon as a burial place for horses and the races had to be discontinued, other factors also contributing. Now there are not more than half-a-dozen well-bred horses left in the whole of Jamshedpur.

The following table compiled from information gathered locally will show the losses from this disease at Jamshedpur during the last few years :—

| Year           | No. of cases   |
|----------------|--|
| 1925 . . . . . | 1  |
| 1926 . . . . . | Nil.   |
| 1927 . . . . . | 3  |
| 1928 . . . . . | 5 (Out of 6 horses that came from Calcutta developed the disease within a few days of their arrival and one succumbed after four days of illness, another after 10-12 days and another after a fortnight; the duration of illness in others was probably similar. Only one horse went back). |
| 1929 . . . . . | }  |
| 1930 . . . . . | Races stopped; no cases.   |
| 1931 . . . . . | }  |
| 1932 . . . . . | 3  |
| 1933 . . . . . | 1 (Mounted Military Police).   |
| 1934 . . . . . | Do.  |
| 1935 . . . . . | 10 Do.   |
| 1936 . . . . . | 6 Do.  |
| 1937 . . . . . | }  |

N.B.—Mounted Military Police troop was first posted in 1933.

#### SYMPTOMS

The clinical picture presented and the degree of severity of attack in the outbreak varied widely and ranged from cases which showed but the barest loss of control over the hind quarters to those which were found down in the stall, struggling violently and completely paralysed.

In the majority of cases the onset was slow and insidious. This was very well exemplified by horse No. 70, which, for about a month before the disease was definitely diagnosed, was seen to execute peculiar swinging movements of the hind quarters. While at rest in the stall and completely undisturbed, the animal would swing back slowly and then suddenly becoming conscious of its unnatural posture resume its normal position again, only to repeat the movement after a few minutes. At this stage there was no suspicion at all of any paraplegia, but later the symptoms became well marked leaving little doubt in one's mind as to the actual condition.

In one or two cases only was the onset sudden. This was best seen in horse No. 12 which apparently in the best of health up to the previous afternoon was found suddenly down in the stall at night unable to get up and exhibiting marked restlessness and excitement. The temperature at this time was 102°. With great difficulty the animal was removed to a loose box nearby where the next day the symptoms of nervous excitement became greatly aggravated and it broke through the stall twice breaking the breast bars

After these cerebral symptoms had subsided, the animal developed hemiplegia with a tendency to go round in a circle. The condition of the animal got steadily worse and it succumbed three weeks later.

In most cases, the symptoms of paraplegia were seen to progress only to a point after which they became stationary. There was no loss of appetite or other disturbance of health and such animals would perhaps have lived indefinitely, had they not been destroyed.

It may be stated that while a febrile phase of a few days' duration, prior to the onset of symptoms of paresis, is considered to be a characteristic feature of equine encephalomyelitis of virus origin, in none of the horses which were involved in this outbreak was any rise of temperature at any stage of the disease noted (except in No. 12 already referred to, the result probably of violent struggling and excitement). The outbreak that occurred during the winter of 1937-38 was also characterized by an entire absence of any pyrexial phase, a feature which serves to distinguish these outbreaks sharply from those which occurred at Multan and in Kashmir.

In practically all cases there was some degree of retention of the urine amounting in the worst affected cases to complete suppression. In the majority of cases the urine was definitely thick and gluey due to the presence of a large amount of mucus and this character is of some diagnostic importance.

None of the animals revealed any interference with the function of the anal sphincter.

In one case the paralysis also involved the retractor muscles of the penis which used to hang down out of the sheath.

Swelling of the near hind fetlock was seen in one case, while one case (No. 13) also showed, coincident with the attack of paraplegia, a filaria worm in the near eye which, however, disappeared before any operative treatment could be carried out. Later, this animal went completely blind in this eye, though it recovered from the paraplegia.

A similar worm was also seen in another horse, involved in the 1937-38 outbreak, but its removal failed to bring about any amelioration of the symptoms.

In all the animals the appetite remained normal throughout and though some of them were kept under observation for months, none showed any signs of muscular atrophy or skin lesions, indicative of trophic disturbances. There was no loss of sensation in the hind limbs in any of the animals nor was any hypersensitivity elicited on application of pressure over the loins.

Briefly summarized, the symptoms in the majority of the animals were mainly those of paraplegia of varying degrees combined with the retention, either partial or complete, of urine in the worst affected cases. In acute cases, the brain was also affected, especially in the earlier stages.

## TREATMENT

Routine treatment with potassium iodide, Nux Vomica, arsenic, etc., combined with other symptomatic treatment was carried out in all cases but without any beneficial results. Two horses (Nos. 8 and 13) in which the attack was relatively mild recovered but recovery in these animals may have been spontaneous rather than due to any treatment adopted.

On the assumption that the trouble might prove to be encephalomyelitis (of virus origin), before the results of transmission experiments were known, the treatment of all the affected animals was carried out on the lines adopted by Mosley, Heane and Shirlaw [1934] in the Multan outbreak but without any appreciable benefit. Cases which occurred subsequently were also treated in the same way. This treatment was as follows :—

About a pound of mag. sulph was administered per stomach tube and at the same time the animal was given an enema and catheter passed if there was any evidence of retention of urine. This was repeated as often as necessary. Small quantities of mag. sulph (4 oz.) were given daily on subsequent days. Injections of hexamine in doses of 25 grm. dissolved in about 60 c. c. of water were given intravenously on six consecutive days. Forced exercise was given daily to all animals that could walk.

By the time this treatment could be carried out horses Nos. 12 and 52 had already died. The Commandant's horse, which was the worst affected at the time, showed some improvement in the beginning but later the condition relapsed and the animal had to be destroyed, when it could no longer stand even with the support of slings. In the less severely affected cases also there was no improvement.

Trials carried out with the hexamine treatment in the 1937-38 outbreak showed that the drug is of some value especially in the earlier stages, as a surprisingly quick recovery was obtained in some cases following the administration of this drug, but on the whole it may be stated that once the symptoms have become established and definite degenerative changes have occurred in the central nervous system the prognosis is bad and there is no specific remedy that could be relied upon to effect a cure.

## POST MORTEM FINDINGS

*Post mortem* examination was carried out on nine horses, of which two had died naturally and seven had been destroyed because there appeared no hope of their recovery. They may be classified as three acute and six subacute cases.

Briefly speaking, it may be stated that the *post mortem* findings in all those animals less severely affected were almost of an entirely negative character, but definite lesions were seen in the more acute cases. These consisted of petechial haemorrhages on the serous membranes, on the surface of the spleen, in the endocardium (one case) and on the serous coat of the large intestine,

The liver was found to be fatty in two cases, while congestion and petechial haemorrhages were seen in the kidneys in practically all cases. In all cases in which there was no retention of the urine the bladder was found to be normal, but in those with any degree of suppression of the urine it was either found to be enormously distended or shrunken in size to a small volume, with a greatly-thickened wall due to infiltration with new granulomatous tissue, consequent on the irritation set up by cystitis. In such cases the bladder contained only a small quantity of a thick oily urine of an offensive odour and with a quantity of a thick greenish-coloured sediment composed of precipitated mineral matter adhering to the mucosa, which on its removal was found to be thickly studded with haemorrhages.

Exactly similar changes in the bladder had been seen in horse No. 80 which had been attacked in 1935.

The brains and spinal cords of all the animals that were destroyed were carefully examined but except for a few petechial haemorrhages, here and there, in the cord and some evidence of softening especially in the lumbar region not much in the way of naked-eye changes was seen.

#### HISTOPATHOLOGICAL CHANGES

Pathological tissues from a number of cases were sent to Mukteswar for histological examination. The findings were more or less identical in each case and they may be briefly summarized as follows :—

*Spinal cord*.—Congestion of the meningeal vessels. Marked gliosis, especially of the grey matter. Degenerative changes with perivascular haemorrhages in the grey matter which also showed some degree of infiltration (glia) around the central canal which appeared dilated.

*Cerebrum*.—Congestion of meningeal vessels. Gliosis with microscopic haemorrhages, some of which were perivascular. Neuro-nephrosis. Slight cuffing of blood vessels.

*Kidney*.—Deep congestion. Slight interstitial changes. The tubules showed early degenerative changes, and even necrosis in places. The glomeruli were congested, and showed leucocytic infiltration.

*Urinary bladder (in a protracted case with thickening of the wall)*.—Marked congestion. The mucous membrane highly petechiated and hyperplastic with desquamative and necrotic changes on the free edge. The mucosal surface showed Gram-positive bacteria and deposit of blood pigment, and in some places the entire mucosa had been desquamated. A few foci of lymphocytic infiltration were present in the submucosa.

## DIAGNOSIS, RESULTS OF TRANSMISSION EXPERIMENTS, ETC.

By the time the author arrived on the scene eight horses had already been affected and two of these were in a moribund condition. These died on the subsequent day. The clinical picture presented by the animals, the *post mortem* findings, the course of the disease and the occurrence of so many cases within such a short space of time suggested the possibility of some infective agent at work and equine encephalomyelitis was suspected. Material was at once forwarded to Mukteswar for biological examination as no facilities for such examination existed locally and at the same time hexamine treatment of the remaining horses was started which as already stated proved ineffective.

Meanwhile, other possible causes of the trouble were thought of and in this connection the possibility of vegetable poisoning seemed to merit especial consideration in view of the common belief among the people in the locality that grass obtained from a certain farm was in some way connected with causing symptoms such as were seen in these horses, a possibility which could not be entirely ruled out in view of the well-known existence of certain plants which can exercise a poisonous effect on the central nervous system and the fact that although the Mounted Military Police horses at Jamshedpur are maintained ordinarily on hay, bran and oats with boiled linseed once a week, this year partly with a view to conserving the supplies of hay and partly with a view to supplying some sort of green stuff to the animals of which they are ordinarily completely deprived, these horses had for sometime before the outbreak started been fed on grasses obtained from this particular farm (lucerne, guinea-grass and ordinary grass) and continued to be so fed for sometime afterwards. Chemical examination of the grasses, however, failed to reveal any poison. This was at first regarded as not entirely to exclude the possibility of vegetable poisoning as by the time samples of these grasses were collected, they had so completely changed their texture that it was considered not improbable that any poisons present might have disappeared. In this connection it is interesting to note that though no grass either from this farm or any other source is being fed, the disease has recurred this year (1937-38) among these horses with exactly similar clinical manifestations to those seen before.

Coming to the question of an infective agent, Mukteswar found that of two rabbits and two guinea-pigs inoculated sub-durally with brain material from one of the affected horses, both the rabbits failed to develop the infection while of the guinea-pigs one died after showing paraplegic symptoms and the other survived after a febrile reaction. Similar results had been obtained by Shirlaw [1934] in the Multan outbreak and this led the author to suspect that probably a virus was the etiological factor concerned, but unfortunately Mukteswar was unable to repeat the results when supplied with some further material from two horses later. In this connection, however, it is necessary to mention that this material had been taken from horses which had been affected for about a year and, therefore, the possibility of such material having become avirulent cannot be entirely ignored.

A pony injected by the author locally with 10 c. c. of brain emulsion from a horse that had been destroyed after a severe illness lasting for about three months also failed to develop the disease, while an attempt to infect another pony by injecting it intrathecally and intranasally with 40-50 c. c. of cerebro-spinal fluid taken from horses involved in the 1937-38 outbreak yielded no better results.

It will thus be seen that the results of transmission experiments on guinea-pigs, rabbits and ponies have been too uncertain and inconsistent to justify any definite conclusions about the virus origin of the malady, though in view of the known difficulty of transmitting equine encephalomyelitis experimentally, such a possibility cannot be entirely excluded. Nevertheless, one cannot help thinking that if a virus was the real cause of the trouble the percentage of successes should have been greater than that obtained in the experimental transmission work. It is also well known that in carrying out experiments with brain tissue great care is necessary both with regard to the technique and the material used as otherwise quite misleading results may be obtained.

It is proposed to carry out further experiments when some of the horses involved in the present outbreak (1937-38) are destroyed.

The possibility of a mineral imbalance as a factor in the etiology of the condition has not been overlooked and at the request of the Imperial Veterinary Research Institute, Mukteswar, which is engaged in an investigation of this disease and other similar diseases from this point of view, samples of serum and fodder were supplied for analysis, but the results so far to hand do not reveal any abnormality in the calcium and phosphorus contents of these materials of such a consistent nature as to warrant any definite conclusions. It is proposed to pursue the investigation from this point of view as and when opportunity occurs.

While on this subject, the possibility of cold as a probable factor in the causation of the condition must be mentioned, for it was observed that the majority of the cases in the 1936-37 outbreak (as also those in the 1937-38 outbreak) were among horses tethered on the side of the stables exposed to the cold westerly winds, while very few cases were seen among horses tied on the other side. It is not improbable that cold acts as an important predisposing factor in the causation of the condition, tending to lower the vitality of the animal and rendering it susceptible to attack by the specific cause whatever that might be. Support is lent to this view by the fact that in both the outbreaks which have occurred at Jamshedpur so far the disease has shown a definite seasonal incidence. The first cases are usually seen to arise about October or November and the disease tends spontaneously to subside about February or March with the onset of the warmer weather. The outbreaks at Multan and in Kashmir also occurred during the colder part of the year though the exact relationship between them and the disease in this Province has not

been established. It must, however, be admitted that the greater incidence of the disease at this time of the year may be explained on other grounds as well.

#### DISCUSSION AND CONCLUSION

In the foregoing, an account has been given of an outbreak of equine encephalomyelitis among the Mounted Military Police horses at Jamshedpur in the year 1936-37 supported by brief references to another outbreak in 1937-38, and of the steps taken to establish a diagnosis. The possibility of a virus, of vegetable poisoning and of mineral imbalance in the causation of the condition has been discussed but the results have either been wholly negative or not sufficiently definite to justify one in pronouncing a definite opinion. Certain points of resemblance between this outbreak and those at Multan and in Kashmir have been stressed. At the same time attention has been drawn to the important points of difference between them, such as the absence of a definite pyrexial phase in the outbreaks in this Province, the almost negative results of transmission experiments, and the disappointing results obtained with hexamine treatment which was reported to have given good results in the Multan and Kashmir outbreaks, especially in the latter. It is not improbable that the encephalomyelitis occurring in this Province is not a virus malady at all, but one due to some other cause.

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# DISEASES TRANSMITTED BY THE INDIAN SPECIES OF TICKS AND THE POSSIBILITY OF THEIR PREVENTION THROUGH BIOLOGICAL CONTROL.\*

BY

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## INTRODUCTION

COMPARED with what is known in other countries about tick-borne diseases, very little appears to be on record in India. As to the amount of loss due to tick-borne diseases in India there is no record. In a country, where no importance is attached to the loss of human life through diseases transmitted by arthropods, one should not expect any consideration for cattle and other domestic animals. This article is not intended to be an exclusively original contribution, but it is put forward in the hope of enlisting further active interest in these parasites and the diseases which they transmit in India. India offers a rich field for investigators to study the various aspects of tick-borne diseases which have hitherto remained unsolved, and which are unquestionably of vital importance to the stock-breeders.

The tick fauna of India as in other tropical and sub-tropical countries is very rich and is represented by eleven genera and about seventy species. They are found on all kinds of domestic and wild animals like mammals, birds, snakes, lizards and tortoises, and they also attack man. Economically nine species listed in Appendix A are of considerable importance. They attack the domestic animals in India in large numbers and thus they are the chief live-stock pests. In addition there are twenty-six species listed in Appendix B, which have been found on domestic animals but nobody knows as to the importance of the rôle they play in the economy of domestic animals in nature. Very little is known as to the life-history of even the commonest species and the rôle they play in the spread of diseases in India.

The number of Indian species is larger than that which is recorded from the United States of America, where about forty species have been listed so far [Bishopp, 1935]. In the latter country the tick problem attracted attention as early as the last decade of the nineteenth century; with the result that a large number of workers took interest in ticks, and with the increase of the knowledge of them a progressively large number of tick-borne diseases began to be known. Keeping in view, the ecological conditions

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of India which are more favourable to the increase of the population of ticks, and the larger number of the Indian species, the present author is of the opinion that the tick problem is as, if not more serious in India than it is in the United States of America, where an enormous amount of money is spent every year upon the control of ticks alone.

The following is an attempt to review the existing information about the diseases transmitted by species of ticks found in India, but as the records of tick-borne diseases from India are few, the author has incorporated all those cases which have been reported from outside India, as there can always be a possibility in a vast country like India of some of the diseases being never brought to the notice of competent persons. With the rapid mode of transport like aeroplanes there is always a possibility of the transference of infected ticks from outside, as they can live without food for a long time. According to Nuttall [ 1908 ], "Ticks imported from India and Africa have transmitted the disease to dogs in England, and ticks imported from Africa have transmitted piroplasmosis to cattle in England." There is every possibility of the fresh introduction of some of the diseases in India.

#### DISEASES TRANSMITTED BY INDIAN TICKS

The importance of ticks in general as transmitting agents of various diseases of man and domesticated animals continues to become more manifest as our knowledge of them increases. In man they transmit the causative agents of the relapsing fever (spirochaetosis) all over the world, the rickettsial diseases like Tick typhus fever, Marseilles fever, and the Rocky Mountain spotted fever of America and Tularaemia. In domesticated animals they transmit diseases caused by *Babesia* Starcovici (=*Piroplasma* Patton), *Theileria* Bettencourt, França and Borges, *Anaplasma* Theiler, *Spirochaeta* Ehrenberg, *Bacteria* and *Viruses*.

*Piroplasmosis*.—Of the diseases which ticks transmit the diseases due to *Babesia* are amongst the most devastating which affect domestic animals all over the world. In many parts of the world it is impossible to import cattle for the reason that as many as 90 per cent may die due to piroplasmosis. According to Bishop [ 1935 ], in the United States of America the estimated loss from Texa fever in cattle amounts to 40 to 100 million dollars a year. The Texa or tropical Red-water fever of cattle (bovine piroplasmosis) due to *Babesia bigemina* (Smith and Kilborne) is transmitted in India by *Hyalomma* (*Hyalomma*) *aegyptium* (Linnaeus) and *Boophilus australis* (Fuller). According to Cooper [ 1926 ], this disease is widely distributed in India but in most localities it infects young calves which on account of a great power of resistance recover from the disease and consequently they become "immune by virtue of being 'carriers' of the parasite in a latent state of activity for the rest of their lives." Thus to a greater extent this disease is harmless in India but it manifests itself in this country when the infection is transmitted in adult cattle especially when they are imported into India from countries where this disease is not found. The disease also results

when the immunity, obtained by cattle as young calves, is broken down, such as is liable to occur through the effects of intercurrent disease condition due to for example, rinderpest (P. 358) or exposure to adverse conditions'. According to Sturges [ 1929 ], this disease is very common in Ceylon amongst low-country cattle. Severe symptoms are rare except when cattle are weakened by food shortage, etc. *Haemaphysalis bispinosa* Neumann, a species very common in India, is incriminated to transmit this disease in Australia [ Fielding, 1926 ].

*Babesia bovis* Starcovici the causal agent of the European Red-water fever is transmitted in all the countries of Europe by *Ixodes ricinus* (Linnaeus) and in some European countries by *Haemaphysalis cinnabarinus* var. *punctata* (Canestrini and Fanzago) also. Both these species have been recorded from the Western Himalayas but the disease according to Cooper [ 1926 ], has not been reported from India. The mortality due to this disease varies from 6 to 60 per cent. According to Sergent, Donati, Parrot and Lestocquard [ 1928 ], *B. berbera* (Sergent, Donati, Parrot, Lestocquard, Plantureux and Rougebieuf) is transmitted by *Boophilus annulatus* subsp. *calcaratus* (Birula) which is found in India, in Algeria. The piroplasmosis of sheep and goat is due to *Babesia motasi* Wenyon and *B. sergenti* Wenyon. The disease due to *B. motasi* is dangerous and has been reported from Mysore State by Achar and Srikantiah [ 1934 ] in sheep which were infested with *Haemaphysalis bispinosa*. They found this parasite normally non-pathogenic in some sheep but it became pathogenic during the attack of rinderpest. *B. sergenti* was found in the blood of goats in India by Krishna Iyer [ 1932 ]. It produces no recognizable symptoms in sheep and goats.

The equine piroplasmosis or biliary fever in India is due to two species, *Babesia caballi* (Nuttall) and *B. equi* (Laveran). According to Valladares [ 1914 ], both of them are possibly transmitted in India by *Hyalomma (Hyalomma) aegyptium*. *B. equi* is found in mules and donkeys and Lingard and Jennings [ 1904 ] have reported this parasite from camels in India.

The disease known as malignant jaundice of dogs is caused by *Babesia canis* (Piana and Galli-Valerio) and has a wide distribution in the old world including India. It frequently terminates fatally. *Rhipicephalus sanguineus* (Latreille) transmits this disease in India and *Haemaphysalis leachi* (Audouin), which is also found in India, in Africa. *B. gibsoni* (Patton), which was first reported from the Madras hounds, produces infection in jackals and dogs. Sen [ 1933 ] suggests the possibility of its transmission by *R. sanguineus*.

Carpano [ 1929 ] suggests that piroplasmosis in fowl due to *Egyptianella pullorum* Carpano is transmitted by *Argus persicus* (Oken), a species found in India, in Egypt.

*Theileriosis*.—Amongst the diseases caused by *Theileria*, the disease known as the East Coast fever or Rhodesian fever which is due to *Theileria parva* (Theiler) is the most dangerous. The mortality rate due to this disease is as high as 95 to 100 per cent. Wenyon [ 1926 ] includes India in the list of countries from where this disease has been recorded, but no other worker has ever recorded its presence in this country.

A benign type of theileriosis, due to *T. mutans* (Theiler) is widely distributed in the warmer countries of the world. According to Cooper [1926], it is almost universally present in India. It is harmless, but under certain conditions especially when it infects cattle from regions considered to be free from theileriosis it has proved fatal. Infection due to this in adult cattle proves fatal only in 5 to 10 per cent cases. Both *Hyalomma (Hyalomma) aegyptium* and *Boophilus australis* are incriminated in India to be transmitters of this disease. According to Sen and Srinivasan [1937], the causal agents of the acute or fatal theileriosis in India are two forms. One is closely allied to *T. annulata* (Dschunkowsky and Luhs) and other to *T. dispar* Sergent, Donatien, Parrot, Lestoguard, Plantureux and Rougebief. The first form according to them is exotic in origin, and the second one is indigenous and was responsible for a mortality rate of nearly 76 per cent in bulls under experimental conditions. A dangerous parasite *T. hirci* Dschunkowsky and Urodschevich is found in sheep and goats of India [Sarwar, 1935 and Bhatia, 1936]. According to Rastegaieff [1935, 1936, 1 and 1936, 2] *Ornithodoros lahorensis* Neumann can transmit *T. ovis* (=recondita) in sheep of Azerbaijan.

*Anaplasmosis*.—The disease known as anaplasmosis or genuine gall-sickness which is due to *Anaplasma marginale* Theiler is very dangerous and has a fairly wide distribution. The death rate in cattle is 95 per cent in adults, and 50 per cent in young ones. This disease is transmitted by *B. australis* in the Philippines [De Jesus, 1935] and Queensland [Mulhearn, 1936]. Rees [1930] succeeded in transmitting this disease through the agency of *R. sanguineus*, and he [1934] also mentions *I. ricinus* as its vector. In the North Caucasus, *B. annulatus* subsp. *calcaratus* transmits *Anaplasma rossicum*. [Yakimoff, Belawine, Rastegaieff and schlüppikoff, 1929] *O. lahorensis* has been suspected by Rastegaieff [1935, 1936, 1, 2], as the vector of *Anaplasma ovis* in sheep of Azerbaijan. Anaplasmosis is known to occur in India \*.

*Rickettsiosis*.—Among the Rickettsial diseases the Tick typhus fever in man has been reported for the first time from India by Megaw [1921]. Sporadic cases of this disease have often been recorded after that from hilly regions adjoining forests. It has been recorded from Kumaun Hills, Central India Plateau and Orissa forests. *R. sanguineus* was often mentioned as the vector of the disease. Reo [1929] suspected *Rhipicephalus haemaphysaloides* Supino and *Haemaphysalis bispinosa* Neumann. In Sumatra, Kouwenhaar and Wolff [1934] were able to produce rickettsiosis experimentally in guinea-pigs by inoculating suspensions of crushed examples of *Dermacentor auratus* Supino and *R. haemaphysaloides* collected from wild pigs. The present author has identified on numerous occasions for Dr. C. Strickland of the School of Tropical Medicine and Hygiene, Calcutta, nymphs of *D. auratus* collected from man. These resemble *R. sanguineus* to a great extent in outward appearance and can easily be mistaken for *R. sanguineus* by any person

\* *Vide Annual Report of the Imperial Institute of Veterinary Research, Mukteswar for the year ending 31st March 1933, pp. 30, 31.*

who is not well versed in ticks. The present author had since long suspicion that *D. auratus* is the real vector of the Tick typhus fever in India and *R. haemaphysaloides* may play some part in the transmission of the disease.

Marseilles fever (la fièvre boutonneuse), whose causal agent is *Rickettsia conori* Brumpt, is transmitted by *R. sanguineus* [Brumpt, 1930] and has been reported from several places on the Mediterranean coast. The tropical Tick typhus fever in Kenya according to Roberts [1935] is also transmitted by *R. sanguineus*. This disease according to Symes and Roberts [1936] is identical with Marseilles fever.

Rocky Mountain spotted fever which is due to *Dermacentor variabilis rickettsi* (Wolbach) is present in thirteen states of the United States of America, where according to Cooley [1932], more than 4,260 persons have suffered up to 1928 from this disease with an average mortality of about 17·11 per cent. According to Parker, Philip and Jellison [1933] the transmission tests of this disease with *R. sanguineus* over a period of two years have shown that it is a very efficient vector under experimental conditions.

*Spirochaetosis*.—The tick-transmitted spirochaetosis in man causes relapsing fever. *Ornithodoros savignyi* (Audouin), which is almost certainly the vector of the relapsing fever due to *Spirochaeta duttoni* Novy and Knapp in Somaliland and Abyssinia, is found in India. The causal agent of the Central Asiatic [Khodukin and Sofiev, 1932] and Persian relapsing fever is *Spirochaeta persica* Dschunkowsky and its chief vector is *Ornithodoros papillipes* (Birula) though according to some *O. lahorensis* can also transmit this disease. Sergent and Levy [1935] found that *R. sanguineus* can transmit experimentally and in nature in Algeria the Spanish relapsing fever due to *Spirochaeta hispanica* de Buen.

Spirochaetosis of domestic fowls which is due to *Spirochaeta anserina* Sakharoff is transmitted by *Argas persicus* all over the world. It is a very fatal disease and it may kill all fowls in a yard in a few days. According to Rajagopalan [1936] this disease is very common in India.

*Spirochaeta theileri* (Laveran) which is non-pathogenic is found in cattle of India [Lingard, 1907]. Brumpt [1919] succeeded in infecting cattle in France with this species by feeding *Boophilus microplus* on them from Brazil. In the North Caucasus *B. annulatus* subsp. *calcaratus* transmits it [Yakimoff, Belawine, Rastegaeff and Schlüppikoff, 1929].

*Bacterial diseases*.—Tularaemia or Rabbit fever a disease due to *Pasteurella tularensis*, has been recorded from the United States of America and Japan. It is primarily a disease of wild rodents but human infection is of purely accidental nature. According to Parker [1934] the causal agent can survive from larva to adult in *R. sanguineus*.

According to Basu [1930], the chicken cholera or fowl plague due to *Pasteurella avicida* can possibly be acquired by eating infected individuals of *A. persicus*. Faddeeva [1932] obtained 23·6 per cent success in transmitting

plague experimentally by *A. persicus* at Saratov. The causal agent remained alive and effective up to 110 days in the body of the tick.

**Tick paralysis.**—According to some observers the salivary secretion of ticks contains toxins and cases of tick paralysis in some animals are generally explained on the basis of their presence. Tick paralysis has been reported from the United States of America and Australia both in man and domesticated animals. The ticks attach themselves to the body usually on the base of the head or along the spinal column which results in paralysis which progresses upwards and if the tick is not removed the end is fatal. *Ixodes holocyclus* Neumann, which is found in India, causes extremely common and fatal paralysis of dogs, cats, calves, lambs and children in Australia.

According to Regendanz and Reichenow [ 1931], the poison causing tick paralysis is especially formed in the female of *I. sanguineus* during the process of egg development. They experimentally showed that injections of eggs or ovaries of this tick just before oviposition gave rise in dogs to symptoms similar to those of tick paralysis. Mlinac and Oswald [ 1936 ] working in Jugoslavia showed that similarly injections of extracts of eggs of *H. (H.) aegyptium*, *I. ricinus*, *Haemaphysalis cinnabarinus* var. *punctata* can cause tick paralysis. According to Rastegaieff [ 1936, 1] *O. lahorensis* caused fatal tick paralysis in a sheep in Russia.

**Virus diseases.**—Amongst the virus diseases the louping-ill according to MacLeod and Gordon [ 1932 ] is transmitted by *Ixodes ricinus* in England, Scotland and Wales. This tick also transmits the causal agent of the "Tick-borne fever of sheep," [ MacLeod and Gordon, 1933 ] in sheep, pigs and goats.

Rinderpest, a virus disease, which causes 35 per cent mortality of cattle in India, although there is no positive proof of its transmission by ticks, yet it may possibly be transmitted by ticks as it has been shown by De Souza [ 1924 ] that specimens of *Boophilus annulatus* (Say) which had engorged themselves upon an animal infected with rinderpest maintained the virus at full virulence for seven days. In view of this, there is a possibility of the direct transmission of the disease by ticks. In India as pointed out before (p. 355) its complication with piroplasmosis may increase the percentage of mortality.

To give an idea of the extent to which the species of ticks found in India can be concerned in transmission of diseases, the author has listed in Appendix C those Indian species which have been incriminated to transmit diseases either in India or outside India.

#### BIOLOGICAL CONTROL.

There is no doubt that ticks are comparatively free from the attacks of parasites and predaceous enemies on account of their passing a portion of their life concealed within the fur, feathers or scales of their hosts. Unfavourable climatic conditions are mainly responsible for keeping their number in check. Ticks have natural enemies both predaceous and parasitic. The study of

their effects in limiting the increase of population of ticks will yield fruitful results. Regarding the effects of predaceous animals on ticks no organised and systematic attempt has ever been made except here and there one comes across names of a few birds which partially feed on ticks. As regards the parasitic enemies a serious attempt by highly trained persons is being made at present at Montana where a research laboratory costing about sixty thousand dollars was specially built for this purpose in 1927 [ Cooley, 1929, 3 ]. To quote Cooley [1932] "At the present time the use of tick parasites seems to offer the most promising method". So far the parasites of ticks that we know of, all have been discovered by chance in connection with other work. Special efforts should be directed at finding the enemies of ticks.

De Jesus [1934] found experimentally that gordura grass (*Melinis minutiflora*) has a distinctly repellent effect on the larvae of *B. australis*. They die in forty to sixty days on the blades and fifteen to thirty days on the leaf sheaths. He believes in the possibility of producing a tick-free pasture by planting gordura grass and allowing ninety days to elapse after grazing of infected cattle.

*The predaceous enemies of ticks.*—The most efficient predaceous enemies of ticks are found among the birds. According to Newstead [1909], Trinkling Grackle *Quiscalus crassirostris* and Parott-billed black bird *Crotophyla ani* Linnaeus eat ticks specially *B. australis* in Jamaica. Six examples of the former bird were found to contain 159 ticks in their alimentary canal. Bequaert [1930] gives a short review of the tick-eating birds in various countries. Moreau [1933] found 2,291 ticks in the stomach of the red-billed ox-pecker or tick-bird *Buphagus erythrorhynchus* in East Africa. According to De Jesus [1936], the Cattle Egret *Bulbulcus coromandus* feeds on *B. australis* on the bodies of cattle in the Philippines. Shringarpur-Shmidt [1935] found, in the Russian Far East, magpie, *Pica pica* destroying a number of ticks on the deer. The domestic fowls feed eagerly on ticks. If kept in the pens swept and clean, they will pick up any ticks which fall from the cattle and the pens in this way can be kept free from ticks.

According to Hooker, Bishopp and Wood [1912], rats and mice feed upon ticks and 'assist in a limited way in destroying the engorged females'. Toads and lizards also feed on ticks. Sautet [1936] records the feeding of *R. sanguineus* by the spider *Tegenaria triangulosa* in Corsica. Dutton and Todd [1905] found that the eggs and young ones of *Ornithodoros moubata* (Murray) were usually carried away by ants and on 'one occasion over two hundred young ticks were carried off in a single night by small ants'. Vollmer [1931] found that the cloth moth *Tineola biselliella* Humm. feeds on living *O. moubata* and *Argas persicus*.

*Parasitic enemies of ticks.*—Among the parasitic enemies of the ticks there were originally three species of the Chalcidoid family Encyrtidae [ Cooley, 1929, 2 ], viz., *Ixodiphagus texanus* Howard, *I. caucasicus* du Buysson and *Huntrellius hookeri* Howard, but according to Gahan [1934] the second species is the synonym of the third. *H. hookeri* has a very wide distribution and it

has been recorded from the United States of America, Mexico, Cuba, France, Southern India [ Cooley, 1929, 2 ], Indo-China, Portuguese East Africa and South Africa. Africa has been considered the original home of this parasite. According to Bequaert [ 1930 ] in France, it parasitises the nymphs of *I. ricinus* and larvae and nymphs of the genera of *Haemaphysalis*, *Rhipicephalus* and *Dermacentor*.

This parasite was introduced for the purposes of biological control of ticks in 1926 [ Cooley, 1927, 1929, 1 ], in Naushan, a small island near Woods Hole under the supervision of Dr. F. Larruscus from the laboratory of Prof. E. Brumpt of Paris. The control of ticks by this parasite was seriously taken up in 1927 by the Montana State Board of Entomology. In 1928 Cooley [ 1934 ] undertook a tour in South Africa in search of parasites of ticks and he found that this parasite attacks readily the nymphs of *Hyalomma* (*Hyalomma*) *aegyptium* subsp. *impressum* in Transvaal and *Haemaphysalis leachi*. The work of control through this parasite is still in progress with promising results in Montana.

Hunter and Hooker [ 1907 ] have bred a species of Phoridae from the eggs of *B. annulatus* in Texas. The present author has also reared a species of Phoridae in 1925 from the parasitised ticks belonging to the species *A. persicus* from Pusa. The specific identity of this could not be made.

#### CONCLUSION

It is a well-known fact that the work of extermination of ticks must be preceded by and be based upon a knowledge of life-histories of ticks, their habits and the manner in which they are affected by the climatic conditions and destroyed by their natural enemies. Such work has been done extensively in the United States of America and some other countries. According to Nuttall [ 1913 ], wherever this type of work has been carried out intelligently, highly beneficial results have been obtained and "large tracts of country in the United States, Australia and Africa have been rendered almost tick-free by these measures".

In the same way, it is possible to control ticks in India. A list of tick-eating birds and other animals may be prepared and a correct estimate of their efficiency in control of ticks may be made. The common crow and maina *Acridotheres tristis tristis* (Linnaeus) have been observed by the author to feed on ticks on cattle. The Indian Cattle Egret, *Bubulcus ibis coromandus* (Boddart) also feeds on ticks. The natural enemies of ticks should be encouraged in every possible way and domestic fowls should be kept in cattle pens.

The possibility of tick control through the agency of parasitic enemies has proved promising. An organised effort for the search of parasites of ticks may be made. *H. hookeri* which as mentioned above is found in India and it may be utilised for the control of ticks on proper lines.

### Appendix A.

LIST OF SPECIES OF TICKS WHICH ARE OF CONSIDERABLE ECONOMIC IMPORTANCE IN INDIA AND ATTACK DOMESTIC ANIMALS IN LARGE NUMBERS, WITH THEIR USUAL HOSTS.

1. *Argas persicus* (Oken), on poultry all over India.
2. *Argas reflexus* var. *indicus* Warburton, on pigeon all over India.
3. *Haemaphysalis bispinosa* Neumann, on cattle, goat and dog (occasionally attacks sheep, horse and cat), practically all over India excluding North-Western Provinces.
4. *Rhipicephalus sanguineus* (Latreille), on dog (occasionally attacks cattle, horse, donkey and goat) all over India.
5. *Boophilus australis* (Fuller), on cattle (occasionally on sheep, goat, horse and rarely on camel); the commonest cattle tick in Burma, Assam, Bengal and the Andamans, but very common along with *Hyalomma (Hyalomma) aegyptium* in Bihar, Orissa, the Central Provinces, Madras Presidency, the southern districts of the Bombay Presidency, and all along the southern slopes of the Himalayas.
6. *Hyalomma (Hyalomma) aegyptium* (Linnaeus), on cattle, horse, camel, donkey and sheep (occasionally on dog); the commonest cattle tick in the Punjab, Sind, Rajputana and the United Provinces, but in Bihar, Orissa, the Central Provinces and in the Madras and Bombay Presidencies very common along with *B. australis*.
7. *Hyalomma (Hyalomma) aegyptium* subsp. *isnaci* Sharif, on cattle, goat, sheep and dog (occasionally on horse and camel) practically all over India excluding Bengal, Assam and Burma.
8. *Hyalomma (Hyalommina) huassaini* Sharif, on cattle, (occasionally on dog, horse and goat) in Bihar, Central and Peninsular India.
9. *Hyalomma (Hyalommina) kumari* Sharif, on goat, (occasionally on horse, cattle and sheep) in the Northern and Central India.

### Appendix B.

LIST OF INDIAN SPECIES WHICH ARE FOUND ON DOMESTIC ANIMALS, BUT ARE OF COMPARATIVELY LESS ECONOMIC IMPORTANCE, WITH THEIR USUAL HOSTS.

1. *Ornithodoros savignyi* (Audouin), on camel and sheep in the Peninsular India.
2. *Ornithodoros lahorensis* Neumann, on sheep of hilly regions of the North-Western India.
3. *Ornithodoros papillipes* (Birula), on sheep, camel and poultry in the hilly regions of the North-Western India (occasionally attacks man).
4. *Ixodes ricinus* (Linnaeus), on dog and sheep in the Western Himalayas.
5. *Ixodes acutitarsus* (Karsch), on ox in the Eastern Himalayas (occasionally attacks man).
6. *Ixodes japonensis* Neumann, on cattle in the Upper Burma.
7. *Haemaphysalis cinnabarinus* var. *punctata* (Canestrini and Fanzago), on goat in Chitral.
8. *Haemaphysalis scicelli* Sharif, on goat in the Hazara District, Western Himalayas.
9. *Haemaphysalis sundrai* Sharif, on sheep in Kumaon Hills.
10. *Haemaphysalis montgomeryi* Nuttall, on pony, dog, cattle and sheep in Kumaon Hills and hills of the Central India.
11. *Haemaphysalis flava* Neumann, on dog, cattle (occasionally attacks man) in Kumaon Hills.
12. *Haemaphysalis bispinosa* var. *intermedia* Warburton and Nuttall, on cattle and dog practically all over India excluding North-Western Provinces.
13. *Haemaphysalis turturis* Nuttall and Warburton, rarely on cattle in Kumaon Hills.
14. *Haemaphysalis parva* Neumann, rarely on cattle in the Mysore State.
15. *Haemaphysalis leachi* var. *indica* Warburton, on dog, cattle and goat in India excluding the North-Western Provinces.
16. *Haemaphysalis houlettei* Warburton, on pony in the Rawalpindi District.
17. *Haemaphysalis spinigera* Neumann, on cattle in the Peninsular India.
18. *Haemaphysalis cornigera* var. *anomala* Warburton, on cattle and dog in Bihar.

19. *Rhipicephalus haemaphysaloides* Supino, on goat, sheep, cattle, dog and horse all over India.
20. *Boophilus annulatus* subsp. *calcaratus* (Birula), on cattle and horse in Coorg and Mysore States.
21. *Nosolumma monstrosum* (Nuttall and Warburton), on cattle, dog and horse in Orissa, Bihar, and the Bengal and Bombay Presidencies.
22. *Hyalomma (Hyalomma) aegyptium* subsp. *dromedarii* Koch, on camel in the North-Western India.
23. *Hyalomma (Hyalomma) aegyptium* subsp. *ferozedini* Sharif, on cattle and horse in Bihar.
24. *Hyalomma (Hyalommata) hussaini* var. *brevipunctata* Sharif, on cattle, dog and goat in Bengal, Bihar and Peninsular India.
25. *Amblyomma integrum* Karsch, on cattle (occasionally attacks man) in the Peninsular India.
26. *Amblyomma testudinarium* Koch, on cattle in the Eastern and Peninsular India.

**Appendix C.**

LIST OF THE INDIAN SPECIES THAT HAVE BEEN INVOLVED IN THE TRANSMISSION OF DISEASES NOTED AGAINST THEIR NAMES IN INDIA OR IN OTHER COUNTRIES.

1. *Argas persicus*, vector of spirochaetosis in fowl due to *Spirochaeta anserina* all over the world (very common in India); piroplasmosis in fowl due to *Egyptianella pullorum* in Egypt, proved poor vector under experimental conditions of chicken cholera due to *Pasteurella avicida* in poultry and plague in rodents.
2. *Ornithodoros servignyi*, vector of human relapsing fever due to *Spirochaeta duttoni* in Somaliland and Abyssinia.
3. *Ornithodoros papillipes*, vector of Central Asiatic relapsing fever due to *Spirochaeta persica* in Central Asia and Persia.
4. *Ornithodoros lahorensis*, vector of *Theileria ovis* in sheep and goats of Azerbaijan and suspected vector of *Anaplasma ovis* and Central Asiatic relapsing fever in Central Asia.
5. *Ixodes ricinus*, vector of European Red-water fever due to *Babesia bovis* all over Europe, of louping ill and tick-borne fever of sheep in Scotland, England and Wales, and *Anaplasma marginale*.
6. *Ixodes holocyclus* causes tick paralysis in dogs, cats, calves, lambs and children in Australia.
7. *Haemaphysalis bispinosa*, suspected vector of *Babesia bigemina* in Australia and *B. motasi* in Mysore.
8. *Haemaphysalis cinnabarina* var. *punctata*, vector of *B. bovis* in some European countries.
9. *Haemaphysalis leachi*, vector of canine piroplasmosis due to *B. canis* in Africa.
10. *Rhipicephalus sanguineus*, vector of *B. canis* in India and Marseilles fever due to *Rickettsia conori* in the Mediterranean region and Kenya Colony; suspected vector of Tick typhus fever and *B. gibsoni* in India; vector under experimental conditions of *Anaplasma marginale*, *Pasteurella tularensis* and *Spirochaeta hispanica* (Spanish relapsing fever).
11. *Rhipicephalus haemaphysloides*, vector suspected and under experimental conditions of Tick typhus fever in India and Sumatra.
12. *Dermacentor auratus*, vector suspected and under experimental conditions of Tick typhus fever in India and Sumatra.
13. *Boophilus australis*, vector of tropical Red-water fever due to *B. bigemina* and *Theileria mutans* in India and Australia, and *Anaplasma marginale* in the Philippines and Queensland, and vector under experimental conditions of *Spirochaeta theileri*.

14. *Boophilus annulatus* subsp. *calcaratus*, vector of *B. bigemina* in Southern Europe and Caucasus, *B. berbera* in Algeria, *Anaplasma rossicum* and *Spirochaeta theileri* in the Northern Caucasus.
15. *Hyalomma (Hyalomma) aegyptium*, vector of *B. bigemina*, equine piroplasmosis due to *B. caballi* and *B. equi*, and *T. mutans* in India.

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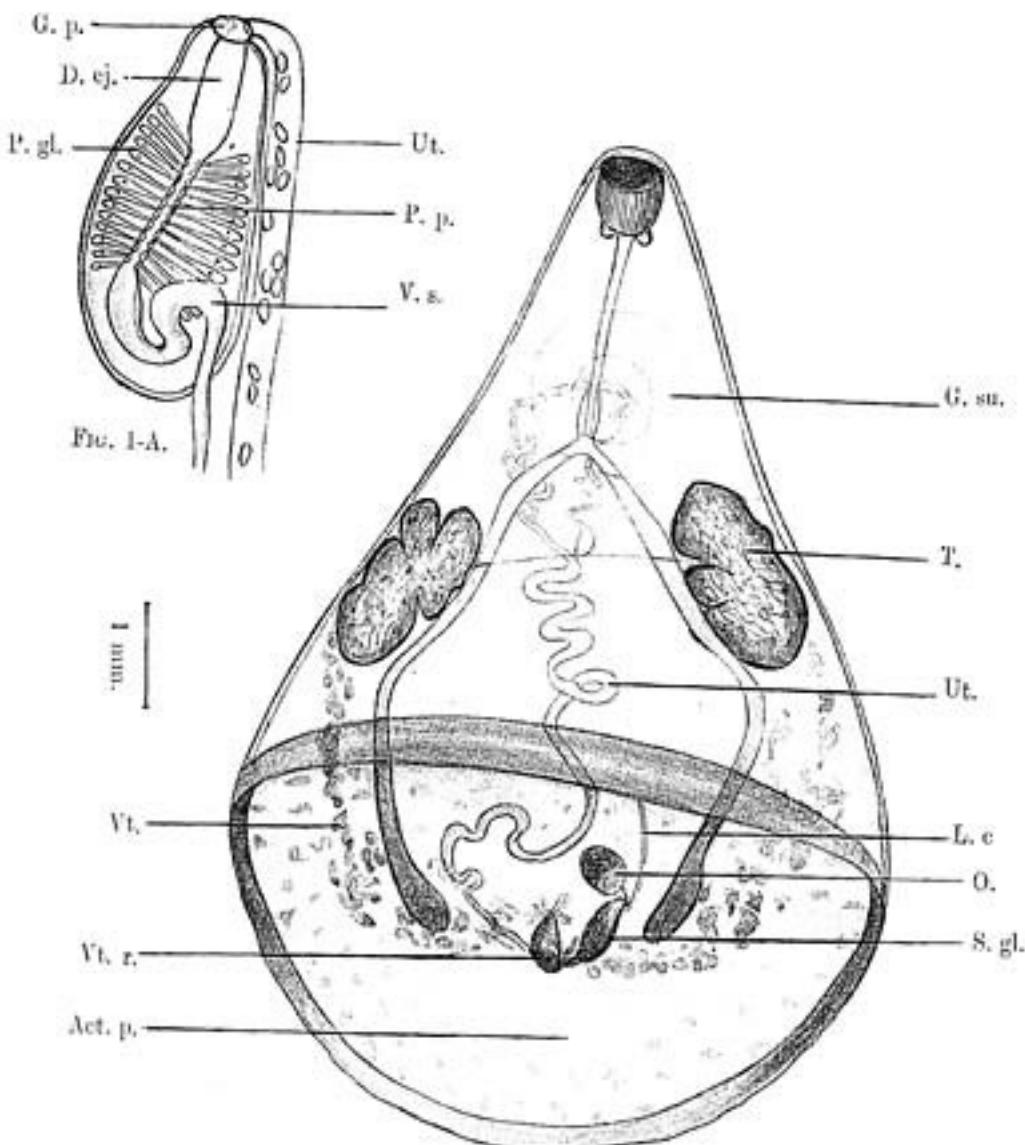


FIG. 1.

FIG. 1. *Nicollodiscus gangelticus*, gen. et sp., nov.FIG. 1-A. Cirrus sac of *N. gangelticus* (enlarged view)*Key to lettering*

|          |    |                            |
|----------|----|----------------------------|
| Act. p.  | .. | Actinophore papillae       |
| C. s.    | .. | Cirrus sac                 |
| D. ej.   | .. | Ductus ejaculatorius       |
| G. p.    | .. | Genital pore               |
| G. su.   | .. | Genital sucker             |
| O.       | .. | Ovary                      |
| P. gl.   | .. | Prostate glands            |
| P. p.    | .. | Pars prostatina            |
| S. gl.   | .. | Shell gland complex        |
| T.       | .. | Testis                     |
| U.       | .. | Uterus                     |
| V. s.    | .. | Vesicula seminalis         |
| V. s. e. | .. | Vesicula seminalis externa |
| Vt.      | .. | Vitellaria                 |
| Vt. r.   | .. | Vitelline reservoir        |

# STUDIES ON THE AMPHISTOMATOUS PARASITES OF INDIAN FOOD-FISHES

PART I—TWO NEW GENERA OF AMPHISTOMES FROM AN INDIAN  
FRESH-WATER FISH, *SILUNDIA GANGETICA* CUV. AND VAL.

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(With Plates XXIX and XXX and three text-figures.)

THE group of digenetic trematodes with a sucker at each end has received considerable attention at the hands of Fischoeder [1901 and 1903], Stiles and Goldberger [1910], Nicoll [1915], Stunkard [1917 and 1925], Poche [1925], Fukui [1929], and Travassos [1934]. Recently, in July 1937, Southwell and Kirchner have described *Chiorchis purvisi* which was collected from a Malayan tortoise, *Heosemys grandis*, by Purvis. The joint authors, after giving a detailed history of the classification of the group, have described the principal characters used by various workers as the basis of classification of this group from time to time. Finally they have proposed a scheme of classification of amphistomes which has been adopted by the author in this paper. In the following pages are described three new trematodes, referable to two new genera, obtained from a common fresh-water fish in the rivers Ganges and Jumna.

Superfamily—Paramphistomoidea Stiles and Goldberger, 1910.

Family—Cladorchidae Stiles and Goldberger, 1910.

Subfamily—Cladorchinae Fischoeder, 1901.

*NICOLLODISCUS GANGETICUS*, Gen. et Sp., Nov.

A number of specimens of this parasite was obtained from the large intestine of a fresh-water fish during the months of July to December 1934. It is a rather rare parasite infesting on an average about five per cent of its host. The number of specimens in a single fish varies from two to twenty. The amphistomes are large in size and are highly muscular. The body is conical in shape with a wide, circular base formed by the posterior sucker. It is completely devoid of spines or scales of any kind, but has well-developed gland cells all over. In permanent mounts the body measures 9·0 to 13·4\* in length and 7·0 to 8·2 in maximum breadth which occurs across the posterior sucker and is nearly equal to its diameter. (Plate XXIX, fig. 1).

\*All measurements are in mm.

The anteriorly directed, cup-shaped oral sucker of  $0\cdot76-0\cdot88 \times 0\cdot76-0\cdot98$  in size opens to the outside through a small vestibule. Posteriorly it bears two small laterally directed pouches, measuring  $0\cdot16-0\cdot5 \times 0\cdot16-0\cdot24$  in size, which open dorsally into it. The posterior sucker is a large, circular structure of  $6\cdot2$  to  $7\cdot5$  in diameter, and is studded on its inner surface with a large number of prominent papillae. It is situated at the posterior end of the conical body forming its base. The oesophagus is a narrow tube,  $1\cdot3-1\cdot4$  long, and has an oval oesophageal bulb,  $0\cdot52-0\cdot84 \times 0\cdot26-0\cdot4$  in size, at its posterior end. The oesophagus and its bulb are both surrounded by numerous deeply staining cells. The caeca follow a typical course and end blindly at the base of the conical body. Their blind ends are slightly swollen. (Plate XXIX, fig. 2.)

The testes are deeply lobed structures situated at about the middle of the body length, one on either side between the caeca and the body wall. The left testis, measuring  $1\cdot54-1\cdot84 \times 0\cdot76-1\cdot02$  in size, is situated a little in front of the right testis of  $1\cdot8-1\cdot9 \times 0\cdot88-1\cdot08$  in size. From each testis is given off a vas efference which runs transversely towards the median line where the two meet to form a common swollen sac. The latter continues anteriorly into a coiled vas deferens which swells up to form the vesicula seminalis on entering the cirrus sac. The cirrus sac is a well-developed, bulb-shaped structure of  $0\cdot34-0\cdot46 \times 0\cdot24-0\cdot28$  in size. It encloses the vesicula seminalis, a small tubular pars prostatica surrounded by prostate gland cells and an elongated, tumbler-shaped ductus ejaculatorius. The male and female pores lie close together in a shallow depression of the ventral body surface, surrounded by a huge but feebly muscular genital sucker,  $1\cdot0$  to  $1\cdot4$  in diameter. The genital pore is slightly sinistral, lying in level with the oesophageal bulb.

The ovary is a small, ovoid body of  $0\cdot46-0\cdot64 \times 0\cdot38-0\cdot52$  in size, situated close to the blind end of the left caecum at the circular base of the body. The elongated sac-shaped shell gland complex lies immediately behind the ovary and measures  $0\cdot5-0\cdot6 \times 0\cdot24-0\cdot36$  in size. The oviduct gives off a narrow fairly long and conspicuous Laurer's canal just before entering the shell gland complex. The vitellaria consist of numerous small follicles of irregular shape and varying sizes, arranged in the form of an U. They begin from the hinder end of testes and extend laterally to the caeca and in the space at the base between the blind intestinal ends. The yolk reservoir lies in the centre of the base. The uterus consists of a single, long, transversely coiled ascending tube containing a large number of eggs,  $0\cdot114-0\cdot152 \times 0\cdot053-0\cdot072$  in size. The uterine coils are confined to the intercaecal space. The excretory system is H-shaped. The lymphatic system consists of three pairs of longitudinal canals. The details of the two systems could not be studied.

The new genus, *Nicolloides*, is named in honour of Dr. W. Nicoll the well-known English parasitologist. It is assigned to the family Cladorchidae on

account of the presence of oral diverticula. Within the family the genus falls under the subfamily Cladorchinae Fischöeder, 1901, and in its affinity stands nearest to *Cladorchis* Fischöeder, 1901. It resembles the latter genus in the presence of a genital sucker but differs in the conical shape of its body, size, position and character of the acetabulum, size of the genital sucker and the position of the gonads.

#### GENERIC DIAGNOSIS

Fairly large sized amphistomes with smooth, conical body. Oral sucker with paired pouches; acetabulum very large, circular, posteriorly directed, papillose and forming the base of the conical body. Oesophagus and oesophageal bulb present; caeca extend to hinder end. Testes, two-lobed, extra-caecal and slightly symmetrical, placed one on either side about the middle of the body. Cirrus sac enclosing vesicula seminalis, pars prostatica surrounded by prostate gland cells, and ductus ejaculatorius present. Genital pore slightly sinistral and in level with the oesophageal bulb. Genital sucker very large but feebly muscular. Ovary compact, situated near the blind end of the left caecum. Laurer's canal present. Vitellaria follicular, arranged in the form of an U, beginning from the hinder end of the testes and meeting posteriorly in the intercaecal space. Uterus pre-ovarian, intercaecal; eggs numerous, operculate and large sized. Excretory system H-shaped; lymphatic system consists of three longitudinal canals. Parasites of fresh-water fishes.

#### TYPE-SPECIES.—*NICOLLUDISCUS GANGETICUS*

#### *ORIENTODISCUS LOBATUM*, GEN. ET. SP., Nov.

Four specimens of this species were collected from the rectum of one out of a number of specimens of the host examined in 1934. The smooth elongated, fusiform body measures 5·0 to 7·0 in length and 1·12 to 1·82 in maximum breadth across the level of the testes. The anteriorly directed oral sucker is ovoid in outline and opens to the outside through a small vestibule. It measures 0·22—0·24 × 0·26—0·3 in size and has two postero-lateral pouches of 0·1—0·12 in diameter. The acetabulum is a strongly muscular, cup-shaped structure of 0·68—0·76 in diameter and is situated sub-terminally at the posterior end of the body. The narrow oesophagus, 0·44—0·54 long, is continued posteriorly into a spindle-shaped oesophageal bulb, 0·3—0·5 × 0·14—0·22 in size. The intestinal caeca are long, narrow tubes with crenated outer margins. They extend in a somewhat wavy course up to the posterior level of the ovary. Both the oesophagus and its bulb are surrounded by a large number of deeply staining cells.

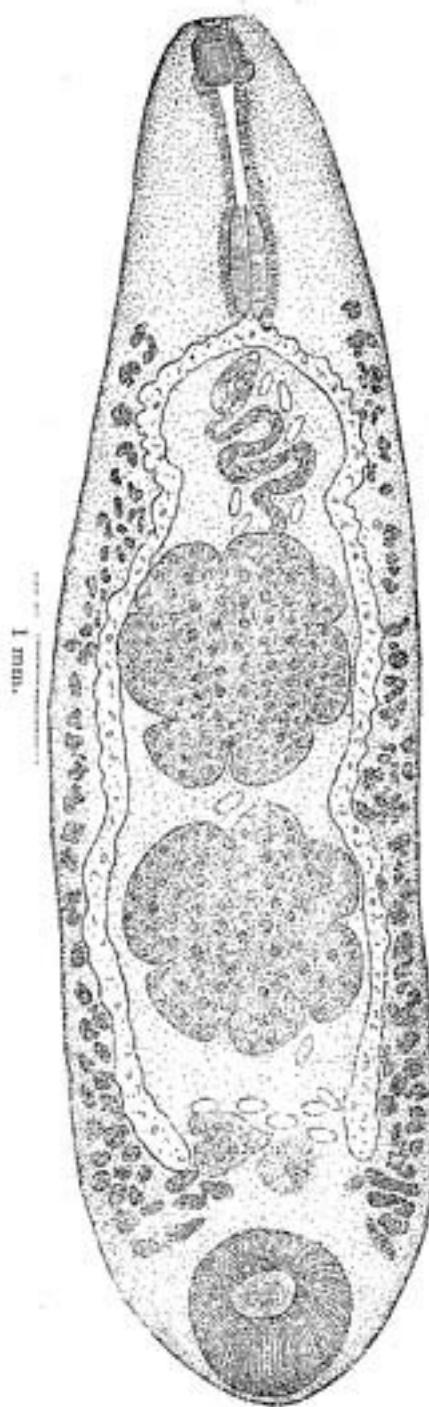


Fig. 1. *Orientodiscus lobatum*, Gen. et Sp., Nov.

The testes, two in number, are lobed, massive structures situated one behind the other in the intercaecal space. The anterior testis, measuring  $0.7-1.04 \times 1.14$  in size, lies at the end of the anterior half of body length. The posterior testis,  $0.64-1.12 \times 1.66$ , is situated behind the anterior testis. The vesicula seminalis is a fairly thick transversely coiled tube divisible into vesicula seminalis interna and externa. The small egg-shaped cirrus sac,  $0.22-0.3 \times 0.12-0.2$  in size, encloses a small portion of the vesicula seminalis, a small tubular pars prostatica surrounded by prostate gland cells and a minute ductus ejaculatorius. The genital pore lies immediately behind the intestinal bifurcation.

The ovary is slightly triangular in shape with a deep notch in its base which is directed towards the blind extremity of the right caecum. It is of  $0.24-0.3 \times 0.22-0.38$  in size, and is situated in the intercaecal space slightly to the right of the median line at one-fourth to one-seventh of body length from the hinder end. A small Laurer's canal is given off from the oviduct. The spherical shell gland mass,  $0.22-0.3$  in diameter, lies a little obliquely behind the ovary. The vitellaria consist of a number of irregular follicles extending laterally to the caeca from the level of the anterior margin of the acetabulum to that of the middle of the oesophageal bulb. At the posterior end they extend a little towards the median line. The uterus consists of a transversely coiled ascending tube containing a number of eggs  $0.114-0.129 \times 0.064-0.076$  in size. The excretory system consists of a pair of lateral tubes which form characteristic loops round the caeca and posteriorly open into the excretory bladder situated dorsally to the acetabulum. The lymphatic system consists of three pairs of longitudinal tubes.

In its affinity the genus *Orientodiscus* stands nearest to *Chiorchis* (as revised by Southwell and Kirchner, 1937). The characteristic feature of the new genus is its excretory system. The anterior extent of the vitellaria also is rather unusual.

#### GENERIC DIAGNOSIS

Body smooth, fusiform; oral sucker with paired pouches; oesophagus, oesophageal bulb and pouches present; caeca extend posteriorly to the level of the ovary; acetabulum strongly muscular, cup-shaped and subterminal. Testes two, massive, intercaecal and tandem; anterior testis situated at the base of the first half of body length. Cirrus sac present; vesicula seminalis divisible into interna and externa. Ovary situated at posterior fourth to seventh part of body length. Laurer's canal present. Vitellaria follicular, lateral, extra-caecal, extending from the level of the oesophageal bulb to the anterior margin of the acetabulum. Uterus intercaecal, pre-ovarian; eggs many and operculate. Excretory system consists of two lateral tubes which form typical loops round the caeca; excretory bladder dorsal to the acetabulum. Lymphatic system consists of three pairs of longitudinal canals. Parasites of fresh-water fishes.

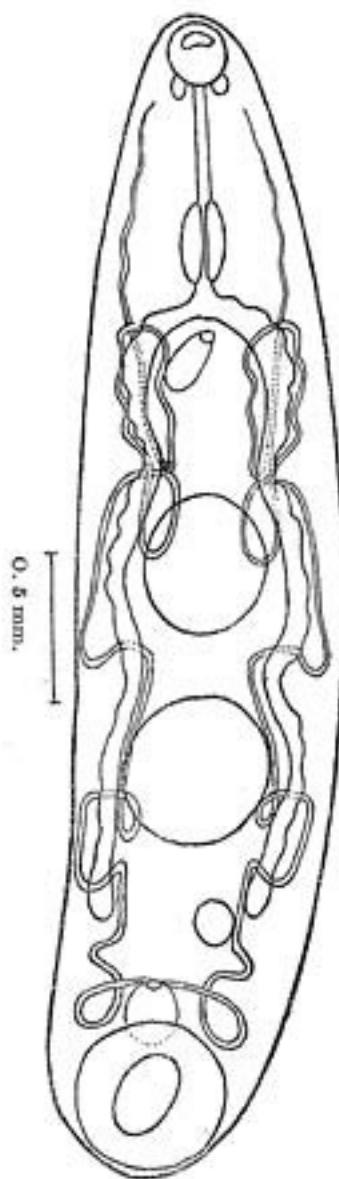


Fig. 2. Excretory system of *O. jumnai*, n. sp.

TYPE-SPECIES.—*ORIENTODISCUS LOBATUM*

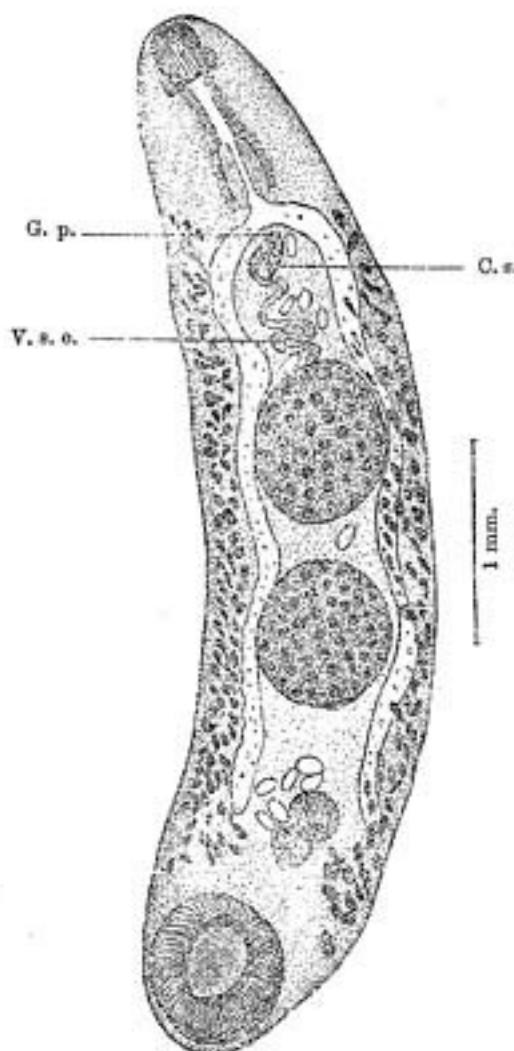
*ORIENTODISCUS JUMNAI*, N. SP.

Six specimens, four mature, of this parasite were obtained from the rectum of a fresh-water fish from the river Jumna in July 1934. The type specimen has



Section of acetabulum of *N. gangeticus* showing acetabular papillae



Fig. 3. *O. jummai* n. sp.

a smooth, cylindrical body of 5·45 in length and 1·2 in maximum breadth in the region of the testes. The ovoid oral sucker 0·24 × 0·26 in size, has a pair of postero-lateral pouches and opens to the outside through a small vestibule. The strongly muscular, cup-shaped acetabulum of 0·66 in diameter is situated subterminally at the hinder end of the body. The narrow oesophagus, 0·32 long, opens posteriorly into an oesophageal bulb, 0·3 × 0·16 in size. The caeca extend in a wavy course up to the level of the ovary.

The two spherical testes are situated in tandem in the intercaecal space in the middle of the body length. They measure 0·66—0·75 in diameter.

A small cirrus sac containing vesicula seminalis interna, pars prostatica and ductus ejaculatorius is present. The vesicula seminalis externa is fairly long and coiled. The spherical ovary of 0·24 in diameter is situated slightly to the left of the median line in the intercaecal space at the beginning of the last quarter of the body length. A small, diffuse shell gland mass lies behind and partly overlapping the ovary. Uterus as in type species and contains several eggs of 0·114—0·12 × 0·072—0·076 in size. Vitellaria are follicular, extending laterally from the anterior level of the acetabulum to the posterior level of the oesophageal bulb. They partly overlap the caeca. The excretory and the lymphatic systems are as in the type species.

*Orientodiscus jumnai*, n. sp. resembles the type species in most of its characters. The only important difference is the unlobed character of the gonads which is a constant feature both in mature and immature forms.

The author is deeply grateful to the Director and the Pathologist of the Imperial Veterinary Research Institute, Muktewar-Kumaun for kind encouragement.

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# OBSERVATIONS ON THE BIONOMICS OF THE OX WARBLE-FLY

(*HYPODERMA LINEATUM DE VILLERS*)

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(Received for publication on 7th March 1938)

(With three text-figures)

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## I. INTRODUCTION

THE present inquiry on the warble-fly infestation in domesticated animals in India was undertaken in January 1937, at the Imperial Veterinary Research Institute, Mukteswar, under a research scheme sanctioned by the Imperial Council of Agricultural Research.

This article deals mainly with some observations made, during a period of about seven months, on the life-history of *Hypoderma lineatum* at Mukteswar, in the Kumaun hills (altitude 7,500 ft.), and its effect on the general condition of the host and also the incidence of infection in one particular type of cattle (hill bulls) in India. Opportunity is also taken for embodying herein a few incidental observations concerning the distribution of the pest in certain localities in the Punjab, in order to illustrate the diversity of habits exhibited by it under varying conditions of topography and climate, for, as will be seen from what has to be stated later, the seasonal occurrence of *H. lineatum* in these localities is not synchronous with what has been observed in the case of this pest in the Kumaun hills. One can hardly over-estimate the importance of observations of this kind in formulating combative measures against the pest.

## II. PERCENTAGE OF INFESTATION IN HILL BULLS

Recent observations have indicated that *H. lineatum* is indigenous to Mukteswar and its neighbouring localities in the Kumaun hills. This is borne out by the fact that during last winter warble tumours appeared in dairy cows

born and bred at Mukteswar, whilst towards the end of April 1937, the writer actually observed what appeared to be an adult *H. lineatum* in a locality adjoining Naini Tal (altitude 5,000 ft.) but which unfortunately he was unable to secure. Additional evidence in support of this statement is provided by the fact that the oesophageal forms of *H. lineatum* larvae have been recovered, during July 1937, from bulls maintained at Mukteswar since October 1936.

In January 1937, a systematic survey was undertaken with a view to obtaining an indication as to the extent of warble-fly infestation in hill bulls at Mukteswar, including those housed in the out-kraals of the station. In all, 372 bulls were examined, out of which 198 (53.23 per cent) showed warble tumours in their backs, the maximum number of warble tumours observed in a single animal being fifty-three. Nearly fifty-five mature larvae were pressed out from some of these tumours and all of these were identified as *Hypoderma lineatum*, which so far has been the only species of *Hypoderma* recorded in this locality.

From the foregoing figures, it would appear that *H. lineatum* is well established in the Kumaun hills, and it would be of interest to enquire into the possible factors that determine the occurrence of this pest at such high altitudes. It is worthy of note that similar observations have also been recorded in the United States of America by Bishopp and his collaborators [1926], for, according to these authors, warble-flies seem to thrive well in fairly high altitudes, this being specially true with *Hypoderma lineatum*, which is known to be abundant at elevations above 7,000 ft. The evidence so far available would seem to suggest that the pest is not influenced—at any rate, to any appreciable extent—by conditions of temperature and topography, as is evidenced by the fact that *H. lineatum* is also abundant in the Punjab which, in respect of both these ecological factors, is very different from the Kumaun hills. The difference in temperature conditions between the two localities may, however, provide an explanation for the fact that while at Mukteswar it was observed that the warble tumours continued to appear in the backs of cattle till early March, at Hissar (Punjab), no tumours were reported to have been observed after the middle of January. It is of interest to note that in the course of a short tour which the author undertook in the Punjab during the winter of 1937, the pest was most prevalent in areas where rainfall was scanty and the soil was sandy loam. The districts of Hissar and Ferozepore were found to be comparatively heavily infested, while, as already recorded by Cross [1926], goat warbles were most common in the "Barbary" breed in the Salt Range area in the Jhelum district.

### III. THE OESOPHAGEAL FORMS

It is of interest to observe that the oesophageal forms of *H. lineatum* larvae have been found to occur at Mukteswar, continuously from the end of March 1937 to the date of writing (October 1937). This is a point which it would seem difficult to reconcile with the generally accepted view that *H. lineatum* has only one brood in a year. The occurrence of these forms has also

been noted in the two neighbouring localities of Naini Tal and Ranikhet during April and May respectively. It may be mentioned that the bulls examined at Mukteswar were brought from Garhwal (altitude 4,000 ft.) about four to six months previously, and that one of the oxen, which showed oesophageal larvae at Naini Tal, was brought from Muzaffarnagar (Western U. P.), while the bulls at Ranikhet were imported from Lucknow only a few days before they were slaughtered. On the basis of the available evidence concerning the length of the different stages in the life-cycle of *H. lineatum*, the oesophageal larvae appearing in the end of March and early April should be the progeny of adult flies that were on the wing in the latter part of January—a conclusion which would seem difficult to uphold, in view of the climatic conditions in Garhwal during this month of the year.

#### IV. THE GRUBS IN THE SUBCUTANEOUS TISSUES

In order to determine with some exactitude the average length of time taken by the larvae to mature after their first appearance on the animal's back, infected bulls were kept under constant observation at Mukteswar. The duration of this period was determined by the interval between the first appearance of the warble tumour and the escape of the mature larva from it.

The following figures (Table I) were derived from eighteen tumours observed on four different bulls :—

TABLE I

| Bull | Number of tumours | Number of days required by the larva to mature in each tumour |
|------|-------------------|---|
| 1    | 8                 | 48  |
| 2    | 4                 | 52  |
| 3    | 4                 | 53  |
| 4    | 2                 | 54  |

At Mukteswar, the average period of development of *H. lineatum* larvae in the subcutaneous tissues of the animal's back was, therefore, nearly fifty-one days.

A perusal of the available literature on warble-flies shows that Laake [1921] is the only worker who has recorded the occurrence of spineless forms of *H. lineatum* larvae in the subcutaneous tissues of cattle. Laake's observations in this respect, however, have been recently disputed by Knippling [1935], according to whom the apparent moulting of an oesophageal larva to a spineless form, as observed by Laake [1921], was merely a process of separation of the outer layer of its cuticle, together with the spiny armature, this process being particularly noticeable in disintegrating larvae. Further he observes that such larvae, being flaccid, are devoid of the characteristics of truly moulting

larvae, and when punctured, yield watery disintegrated tissues. What, however, appeared to be definitely a spineless form of the larva was encountered by the present writer on two occasions in infected animals at Mukteswar. This form shows an entire absence of spines except on the anterior and posterior segments. The cephalopharyngeal skeleton and mouth hooks are morphologically similar to those of the oesophageal stage larva of *H. lineatum*. It differs from the oesophageal larva in being club-shaped instead of torpedo-shaped, whilst the segmentation is more pronounced and the surface less glossy (Figs. 1-3). Unfortunately, both specimens were damaged while being mounted in Berlese's medium.

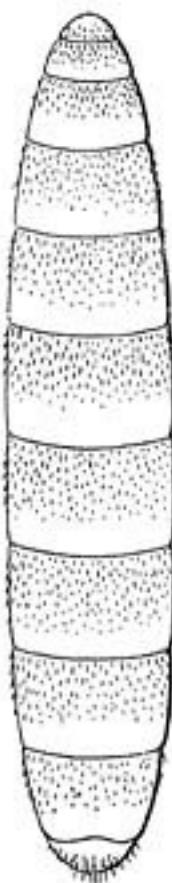


Fig. 1.  
Oesophageal larva,  $\times 11$ .



Fig. 2.  
Spineless larva off back  
of host,  $\times 11$ .

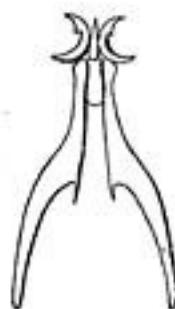


Fig. 3.  
Cephalopharyngeal skeleton as  
observed in both types of  
larvae shown in figs. 1 and 2.

## V. THE EFFECT OF INFESTATION ON THE CONDITION OF THE HOST

Five heavily "warbled" and two warble-free bulls were kept under identical conditions of housing and feeding, and their body weights were taken weekly for nearly three months, from February to April 1937. As will be seen from Table II, the "warbled" bulls continued to lose weight until about the middle of March, which is about the time when the majority of the larvae at Mukteswar escape from their warbles, and from this time onwards they showed a progressive increase in their body weight. No appreciable loss in weight was observed in the two control animals. These observations are in accord with those recorded by MacDougall [1935] in Scotland, who observes that the "condition [of animals] suffers from the presence of a number of larvae in the subdermal tissues".

TABLE II

*Tabulated records showing the loss of body weight in "warbled" animals  
The numbers marked with asterisk represent control (uninfested) bulls.*

| Bull | Weekly body weights in lb. commencing from 5th February 1937 |          |          |          |          |          |          |          |          |           |           |           | Mean weekly loss or gain in body weight calculated upto 6th week when larvae began to drop off. lb. |
|------|--|----------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------|---|
|      | 1st week   | 2nd week | 3rd week | 4th week | 5th week | 6th week | 7th week | 8th week | 9th week | 10th week | 11th week | 12th week |   |
| 1    | 355  | 355      | 345      | 333      | 324      | 320      | 315      | 315      | 312      | 310       | 310       | 311       | -7.0  |
| 2    | 175  | 170      | 170      | 165      | 158      | 152      | 150      | 152      | 153      | 160       | 167       | 188       | -4.6  |
| 3    | 225  | 230      | 230      | 225      | 215      | 212      | 210      | 214      | 223      | 240       | 247       | 248       | -4.6  |
| 4    | 260  | 260      | 240      | 242      | 240      | 235      | 230      | 232      | 232      | 270       | 281       | 290       | -5.0  |
| 5    | 280  | 270      | 270      | 265      | 265      | 260      | 255      | 260      | 260      | 282       | 289       | 297       | -4.0  |
| *6   | 272  | 270      | 270      | 272      | 270      | 275      | 284      | 285      | 300      | 304       | 290       | 296       | +0.6  |
| *7   | 181  | 185      | 185      | 182      | 182      | 182      | 182      | 186      | 182      | 182       | 180       | 182       | +0.2  |

## VI. SUMMARY AND CONCLUSIONS

Observations carried out at the Imperial Veterinary Research Institute, Mukteswar, have shown that:—

Over fifty per cent of the hill bulls purchased for experimental purposes are infested with warble grubs (*Hypoderma lineatum*) and that it takes nearly fifty-one days for the larvae to mature after their first appearance in the subcutaneous tissues of the animal's back. The mature larvae have been encountered as late as the beginning of March, and in this respect the seasonal occurrence of *H. lineatum* at Mukteswar would appear to present a feature somewhat different from that recorded for the same species in certain other localities in India. Thus, at Hissar (Punjab), no tumours in the back are noticed after the middle of January.

The presence of warble tumours in dairy cows and of oesophageal larvae in bulls born and bred at Mukteswar shows that the pest is indigenous to this locality.

During 1937, the occurrence of oesophageal forms of *H. lineatum* was noticed for the first time at Mukteswar towards the end of March and thence-forward they were encountered continuously during an observation period of nearly seven months. The significance of their occurrence over such a long period is not understood and it would appear difficult to reconcile this with the generally accepted view that there is only one annual brood of *H. lineatum* in India.

Evidence has been obtained that the presence of warble tumours results in a loss of condition of the affected animal.

#### ACKNOWLEDGMENTS

The writer wishes to express his thanks to the Imperial Council of Agricultural Research and to the Director, Imperial Veterinary Research Institute, Mukteswar, for providing funds and facilities to undertake this work. His thanks are also due to Mr. S. K. Sen, Entomologist of the Institute, for his useful suggestions and guidance. The illustrations were prepared by Mr. Ahmed Baksh and Mr. S. Sen Roy, artists of the Institute, and for this the writer is indebted to them.

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A STUDY OF THE LIFE-HISTORY AND PATHOGENICITY  
OF *COTYLOPHORON COTYLOPHORUM* (FISCHOEDER,  
1901) STILES AND GOLDBERGER, 1910, OF INDIAN  
RUMINANTS AND A BIOLOGICAL CONTROL  
TO CHECK THE INFESTATION

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(With Plates XXXI—XXXIV)

THOUGH a knowledge of the life-history of a parasite is necessary in determining its pathogenicity and in devising means to check its infestation, the life-histories of only a few trematodes have so far been worked out in this country. Liston and Soparkar [1917] carried out experiments on the life-history of *Schistosoma spindalis* and found that snails of the species *Indoplanorbis exustus* and *Limnaea acuminata* serve as the intermediate hosts. Rao and Ayyar [1932] discovered the molluscan hosts of two amphistomes—*Paramphistomum cervi* and *Fischodierius elongatus*. A year later Rao established the life-cycle of *Schistosoma nasalis*. Bhalerao in 1933 demonstrated that *L. acuminata* serves as the intermediate host of *Fasciola gigantica* in the Kumaun hills.

The occurrence of epizootics of acute amphistomiasis among sheep and goats in the United Provinces was responsible for undertaking an investigation into the life-history of a common amphistome, *Cotylophoron cotylophorum*, of Indian ruminants. Besides establishing experimentally the life-history and the pathogenicity of the amphistome, a biological control to check its infestation has been discovered. It has been observed that while adult amphistomes are apparently non-pathogenic, they are, in their immature stages, highly pathogenic.

Specifically determined adult specimens of the amphistome, obtained from the rumen of goats and sheep, were first thoroughly washed with water and then placed in small beakers containing normal saline and were kept at body temperature in an incubator. When a sufficient number of eggs was laid, the worms, while they were still alive, were removed and the saline was decanted. The eggs were washed in several changes of water. It was found that any bacterial growth in the beaker containing eggs interfered with their development. The eggs were kept in distilled water which was changed at least three

a day. They are whitish in colour and ovoid in shape, with a slight attenuation at the opercular end. They measure  $0.0126-0.013 \times 0.0068-0.009^*$  in size. When kept at a temperature of  $80^{\circ}$  to  $90^{\circ}\text{F}.$ , they hatched in eighteen to twenty-one days. Though hatching of the eggs took place throughout the twenty-four hours, most of them hatched between 10 A.M. to 4 P.M.

The miracidium is extremely active and is attracted towards the lighted side of the container, showing marked heliotropism. It is pyriform in shape and measures  $0.014$  to  $0.02 \times 0.003$  to  $0.005$  in size, with the maximum breadth occurring across the anterior fifth of body length. It is covered, except at the apical papilla, with ciliated epidermal plates which are twenty in number, arranged in four rows round the body. There are a flask-shaped gland (primitive gut), two pairs of penetration glands, reproductive tissue and the usual types of excretory and nervous systems. The miracidia were utilised in infesting parasite-free, laboratory-raised specimens of a common snail *Indoplanorbis exustus*. A few snails were placed in a dish of water containing actively swimming specimens of the miracidia. The larvae attacked the snails very readily and slowly penetrated into its exposed parts, specially foot and mantle. The infested snails began to shed cercariae in thirty to thirty-five days.

#### DEVELOPMENT INSIDE THE SNAIL

The miracidia retain their ciliated plates for a few hours after penetration into the body of the mollusc. Soon they become ovoid or elongated in shape with broadly rounded ends—sporocyst—and the miracidial organs begin to disappear. A very thin cuticle develops round the body. In about twenty-four to thirty hours, the germ balls break up into a number of germ cells which fill the central cavity. The germ cells do not appear to possess cell walls. They develop into rediae which begin to appear in three days' time and attain their full size inside the sporocyst in about a fortnight. The number of rediae in a single sporocyst varies from five to eight. There is no birth pore in the sporocysts and consequently the enclosed rediae come out by rupturing the wall at the anterior end. The pair of flame cells present in the miracidium persist in the fully formed sporocysts which measure  $0.29-0.35 \times 0.12-0.19$  in size.

A fully matured redia is sausage-shaped, measuring  $0.58-0.72 \times 0.14-0.16$  in size, and possesses a mouth, pharynx, oesophagus and a rhabdocoel gut, besides excretory and nervous systems. A number of unicellular bodies are found associated with the gut. There are no lateral appendages. The birth pore develops only after the rediae have emerged from the sporocyst. The excretory system consists of three pairs of flame cells. The cells of the anterior pair lie one on either side of the gut while those of the posterior pair are situated laterally a little in front of the hind extremity. The cells of

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\*All measurements are in mm.



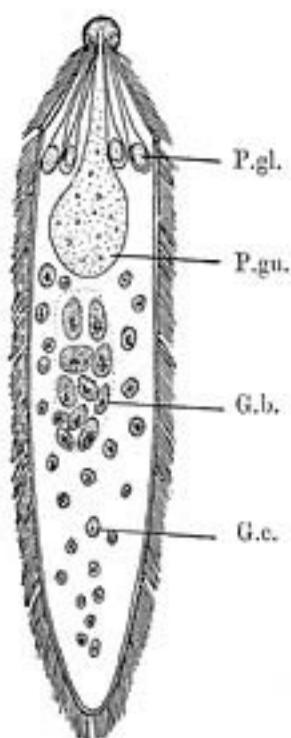


FIG. 1. Miracidium of  
*Cotylophoron cotylophorum*



FIG. 2. Sporocyst of  
*Cotylophoron cotylophorum*

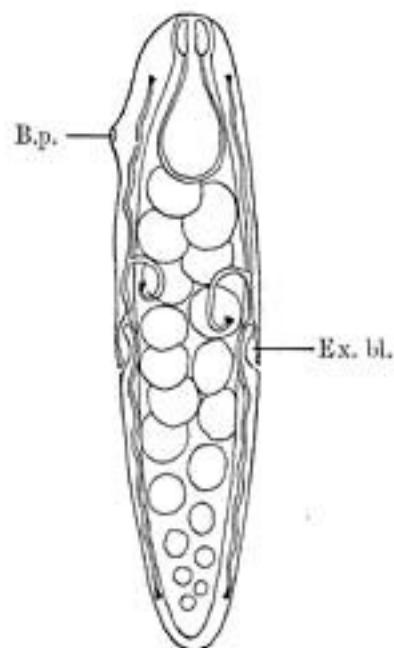


FIG. 3. Redia of  
*Cotylophoron cotylophorum*

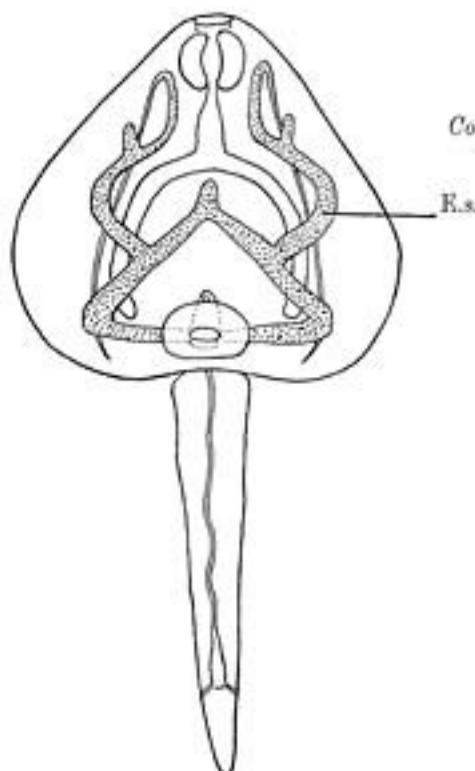


FIG. 4. Cercaria of *Cotylophoron cotylophorum*

Key to lettering

|         |    |    |    |    |                   |
|---------|----|----|----|----|-------------------|
| B.P.    | .. | .. | .. | .. | Birth pore        |
| E.S.    | .. | .. | .. | .. | Excretory system  |
| Ex. bl. | .. | .. | .. | .. | Excretory bladder |
| G.b.    | .. | .. | .. | .. | Germ ball         |
| G.c.    | .. | .. | .. | .. | Germ cell         |
| P.g.    | .. | .. | .. | .. | Penetration gland |
| P.gu.   | .. | .. | .. | .. | Primitive gut     |

Key to references

|                    |    |    |    |    |          |
|--------------------|----|----|----|----|----------|
| Philip Rose        | .. | .. | .. | .. | .9.8     |
| Federated States   | .. | .. | .. | .. | .2.3     |
| Federated Islands  | .. | .. | .. | .. | .Ex. 9.1 |
| Federated Islands  | .. | .. | .. | .. | .4.1     |
| Federated Islands  | .. | .. | .. | .. | .2.5     |
| Gilman Hall        | .. | .. | .. | .. | .3.9     |
| Gilman Hall        | .. | .. | .. | .. | .32.9    |
| Institutionalizing | .. | .. | .. | .. | .32.9    |
| Institutionalizing | .. | .. | .. | .. | .32.9    |



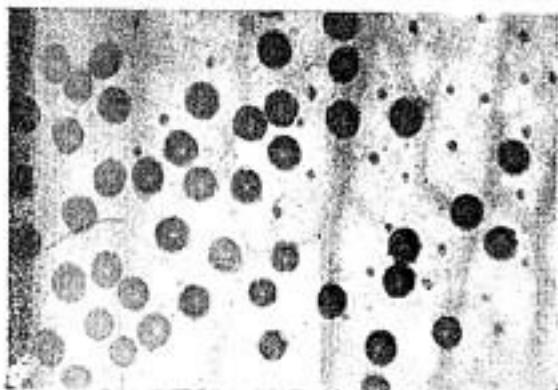


FIG. 1.

Cysts of cercariae of *C. catglaphoratum*

FIG. 3.

Section of duodenum of sheep showing young amphistomes embedded in mucosa (after Cameron)



FIG. 2.

A case of acute amphistomiasis due to *C. catglaphoratum*

the third pair lie one on either side in the equatorial region of the body. The ducts of the third pair of flame cells open into those of the anterior pair. The ducts of the anterior and posterior pairs of flame cells join on either side to form a common duct which swells into a bladder. The excretory pores are situated laterally close behind the middle of body length. The rediae after leaving the sporocyst migrate into the liver and ovotestis of the host where they complete their development. The formation of daughter rediae though not common has been observed in a few cases.

The cercariae belong to the 'pigmentata group'. They leave the rediae through the birth pore in an immature stage and complete their development in the tissues of the host. Fully developed cercariae begin to come out thirty to thirty-five days after the infestation of the snail with miracidia. In their internal structure the cercariae resemble closely *Cercariae indicae* XXIX Sewell, 1922, but differ from it in the size of the body which is smaller and in the presence of a feebly developed envelop of the circular muscle fibres around the base of the oesophagus. Like the miracidia, the cercariae are also strongly heliotropic. They begin to come out in large numbers when the aquarium containing the infested snails is kept in the sun. The largest number of cercariae is shed between 10 A.M. and 4 P.M. After a brief active period, the cercaria contracts into a small, spherical mass around which a thin cyst wall is secreted and the tail is cast off. The cysts are hemispherical in shape and deep gray in colour. The larvae readily encyst on the sides of the container and on suspended vegetation. In these experiments leaves of tamarind and banian trees were provided. A number of leaves bearing large numbers of encysted metacercariae were fed to two out of four parasite-free and laboratory-raised goats, the other two remaining as controls. Four months after feeding the metacercariae the animals showed symptoms of acute amphistomiasis to which one of them succumbed. On post mortem examination numerous immature amphistomes were found embedded in the wall of the duodenum and the initial part of the intestine. The other infested and the two controls were slaughtered six months later and adult specimens of *Cotylophoron cotylophorum* were obtained from the rumen of the first, while the last two were free from amphistomes.

Feeding experiments were carried out to determine the span of life of the encysted metacercariae kept under suitable conditions of temperature and moisture. A large number of leaves containing freshly encysted metacercariae was kept in small moist chambers at room temperature for a period of five months. Parasite-free lambs were given two, three, three and a half, four and five months old cysts and it was found on subsequent examination that the encysted metacercariae remain viable for a maximum period of four months. It was observed that while most of the three months old metacercariae developed in the host only a few out of the four months old cysts could develop into adults.

## PATHOGENICITY

Until recently amphistomes were regarded as perfectly innocuous parasites. While discussing 'Gillar' in his article on 'Some problems in sheep diseases', Baldery in 1906 points out that clinically it somewhat resembles fascioliasis. In describing the pathological lesions he states, "The really pathogenic lesions will be seen in the small and large intestine, noticeably so in the pyloric region of the former. Here there will be seen an enteritis, the mucous membranes being necrotic and probably shredding off in places .... The veins in these areas will have quite a varicose appearance". He collected amphistomes "in an undeveloped state" from the haemorrhagic areas. Though he states, "In subsequent post mortem examinations, the presence of immature amphistomes was found to be constant and always in the diseased duodenum and pylorus", nevertheless he did not suspect these parasites as the causal agents and remarked, "It is supposed that these organisms have not a very serious effect". Speaking of the ovine disease 'Gillar', Walker, [1906], pointed out that it is practically confined to the low-lying swampy areas and that usually the malady is at its worst during the months of December, January and February. Le Roux [1930] investigated two serious outbreaks of amphistomiasis due to *Cotylophoron cotylophorum* in the Orange Free State. In the first outbreak thirty per cent and in the second, fifty per cent of the flock had died. Pande [1935] reported an outbreak of amphistomiasis in cattle in Assam.

On being ingested by suitable hosts the encysted metacercariae excyst in the duodenum and the initial part of the intestine and the immature amphistomes attach themselves firmly to the mucosa. Later, they migrate to the rumen to mature and oviposit. It has been observed by the author that the amphistomes are pathogenic only so long as they are in the duodenum and the intestine and become apparently innocuous on migrating to the rumen. The infested animals in the above experiments appeared dull, weak and anaemic. They suffered from general unthriftiness and persistent foetid diarrhoea till the end. On account of the pronounced anaemia oedema of the submaxillary space, as in fascioliasis, developed. The duodenum and the intestine were found to contain haemorrhagic fluid, and the mucosa, specially of the duodenum, was markedly thickened and necrotic at places. The faeces were often found to be blood-tinged and usually contained large numbers of immature amphistomes. Recovery followed the migration of the flukes into the rumen where they attain maturity and live as practically non-pathogenic adults.

The author did not carry out any therapeutic experiments. Le Roux, [1930] found that carbon tetrachloride in doses of 8 to 10 c. c. in raw linseed oil was effective in case of sheep. However, the large dose recommended by Le Roux is rather dangerous, and should be used with extreme caution. It must not be employed in the case of milking heifers. Instead, tetrachlorethylene and, in the case of cattle, hexachlorethane should rather be tried. The

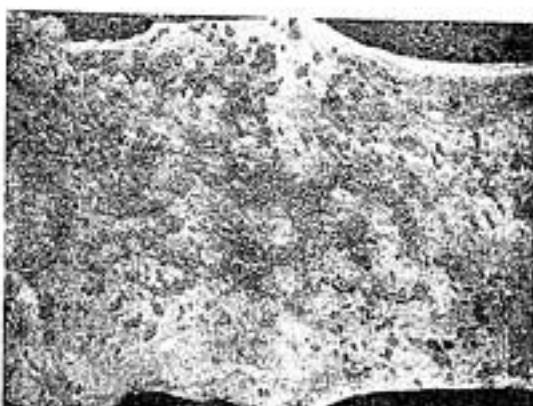


FIG. 1.

Portion of the duodenum showing mass infestation with immature amphistomes (after Le Roux)



FIG. 2.

Portion of the duodenum showing verrucose appearance of the mucosa and whitish raised areas, sites of attachment (after Le Roux)

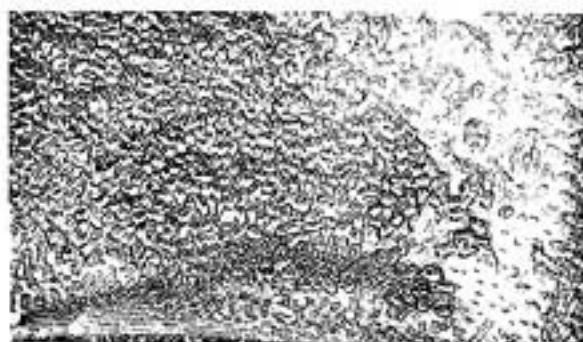
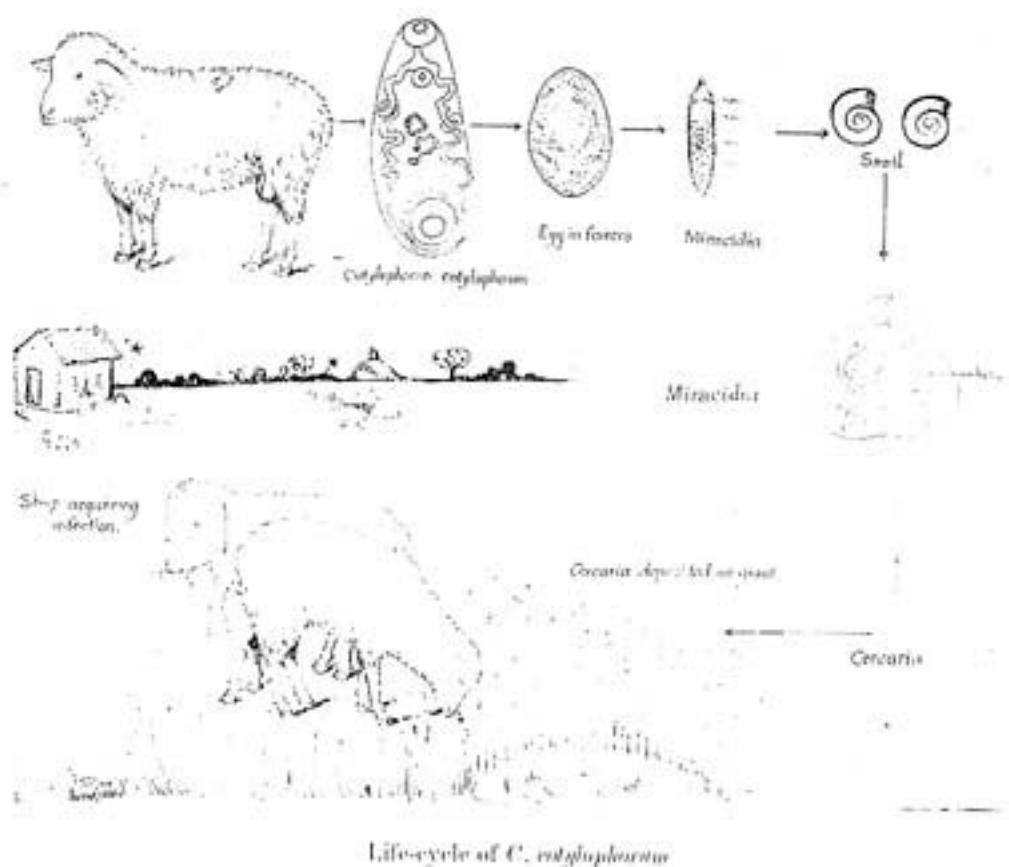


FIG. 3.

Portion of rumen showing *C. rotulophorum* adhering (after Le Roux)



drug should be administered in capsules or by stomach tube to ensure its passage into the rumen. The infested animals should be given bone meal to make up for the loss of calcium. According to various workers administration of magnesium sulphate or calcium lactate reduces the toxicity of carbon tetrachloride and may be tried. Treatment, while there is acute enteritis, is not desirable.

## PROPHYLAXIS

The same as for fascioliasis.

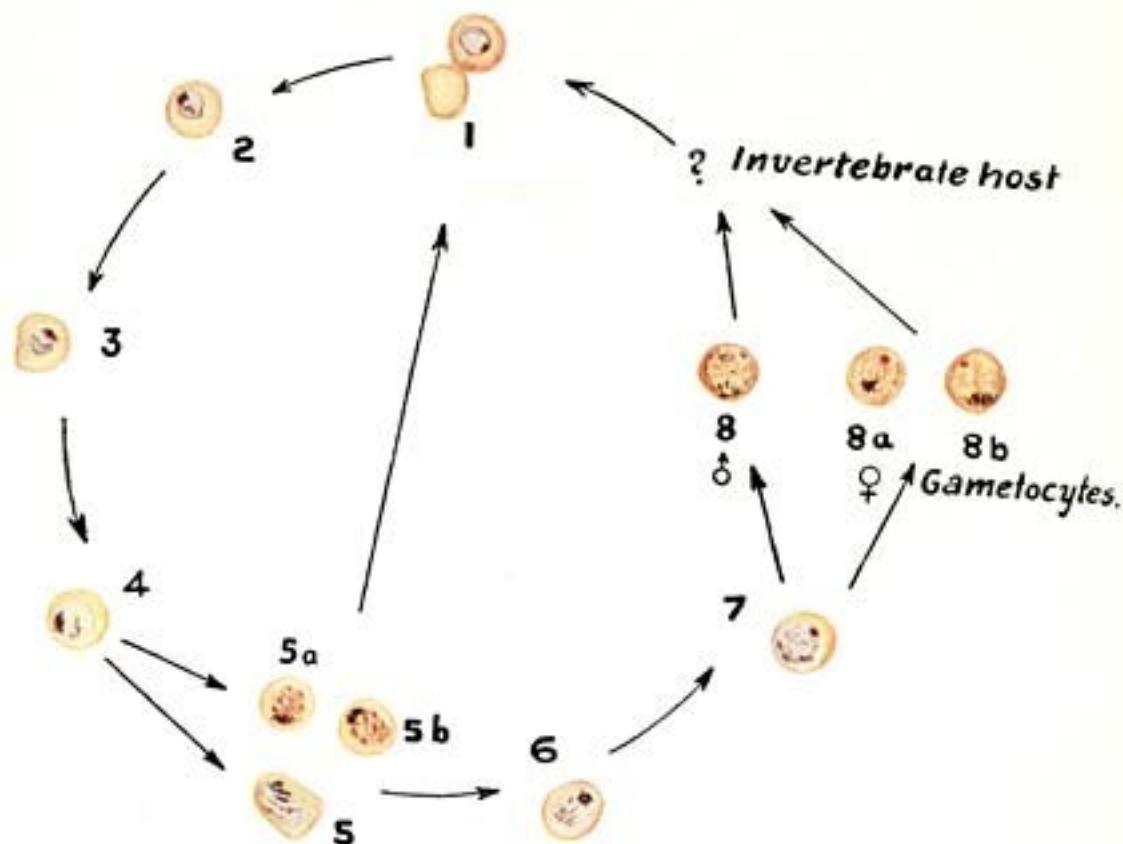
It has been observed that when the snails of the species, *Indoplanorbis exustus*, are infested with an aquatic Oligochaete—*Chaetogaster limnaci*—they can not be infested with trematode larvae, while the same snails when free from the Oligochaete are readily infested with miracidia. Though propagation of the Oligochaete under field conditions has not been tried, the examination of snails from ponds has confirmed the observations made in the laboratory.

The author is deeply grateful to the Pathologist and the Director of this Institute for much kind help and encouragement. He is also thankful to Dr. H. R. Mehra of the Allahabad University for valuable suggestion.

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*Plasmodium bubalis* Sheather 1919  
Schematic diagram of Schizogony



## A NOTE ON *PLASMODIUM BUBALIS* SHEATHER, 1919

BY

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(Received for publication on 19th May 1938)

(With Plate XXXV)

SHEATHER [1919] discovered *Plasmodium bubalis* in the blood of two buffaloes used for the production of anti-rinderpest serum at the Imperial Veterinary Research Institute, Mukteswar. Since then the parasite has been reported from there from time to time. It would appear that this protozoon affecting the buffalo has not been reported from any other province in India excepting Madras. In 1933, the organism was encountered in the blood smears received in the Parasitology section of the Madras Veterinary College from a rinderpest-stricken buffalo near Tekkali, Ganjam district. More material for study could not be had from that animal owing to its death. It was possible to obtain blood smears from other buffaloes from that village but no plasmodium could be detected. In 1936, *P. bubalis* was found by the Superintendent, Madras Serum Institute in the blood smears of ten buffaloes used for anti-rinderpest serum production and one used for serum against haemorrhagic septicæmia. All the eleven buffaloes came from near Gudur, Nellore district. The last animal developed haemoglobinuria and a rise in temperature after the first bleeding. Examination of its blood smears revealed *P. bubalis*. This animal was not used for further bleeding but was kept under observation to watch the natural course of an attack with the plasmodium. But for the rise in temperature and the haemoglobinuria, the animal showed no discomfort and the urine cleared up spontaneously on the following day. The parasites disappeared from the blood as rapidly as they appeared. It seems, therefore, that *P. bubalis* is a cryptic protozoon of buffaloes just as *Trypanosoma theileri* is of cattle, i.e., they show themselves in the presence of an intercurrent disease like rinderpest or when the vitality is lowered by bleeding for the production of serum.

Sheather [1919] has described this plasmodium in its different stages of development in the blood excepting gametocytes, and it is proposed to give below a very brief description of the parasite with a schematic diagram of the schizogony.

The plasmodium which grows in the red blood cells of the buffalo can be conveniently divided into three groups for the sake of description, viz., (a) young ring forms (Merozoites which have entered the red blood cells) (b) developing trophozoites into schizonts and (c) gametocytes.

## (a) YOUNG RING FORMS

The majority of the young forms are ring-shaped but some appear almost like *Babesia*. The young forms measure 1·5 to 2 $\mu$  in diameter. The nucleus is usually seen as a lump of chromatin placed at some part of the circumference of the ring. In some it is crescent-shaped and in a very few, a small dot of chromatin is seen near the larger nucleus.

## (b) DEVELOPING TROPHOZOITES

The ring forms increase in size but seem to show very little amoeboid movement, when they have attained a size ranging from 2·5 to 3·5 $\mu$ , pigment granules appear in their protoplasm. Most of the granules are rod-shaped and of varying sizes. They are usually clumped together in some part of the protoplasm. These granules seem to increase in size as well as in number as the trophozoites grew. A fully grown trophozoite measures 5 to 6 $\mu$  in diameter. Usually the parasitised red blood cell is not enlarged. The blood shows anaemic changes to some extent and it is difficult to ascribe these changes exclusively to the protozoan infection when another disease is also present. Some of the trophozoites which attain the size noted above show signs of division into merozoites. Eight to twelve merozoites have been encountered in schizonts but Sheather [1919] appears to have found seven to fourteen merozoites. These are sometimes seen free in the blood smear and each of these on an average measures 1 $\mu$  in diameter.

## (c) GAMETOCYTES

Some of the full grown trophozoites assume the characters of gametocytes and are about 6 $\mu$  each in diameter. They are rounded in shape and almost fill the parasitised red blood cell. Some of them have their chromatin, more or less compact, near one of the poles and the pigment granules are found aggregated in a mass at the other pole. Its protoplasm as compared to trophozoite shows a tendency to deep staining with Leishman's stain. These are evidently the female gametocytes. The others are slightly smaller about 5·5 $\mu$  in diameter and have their nuclear chromatin loosely gathered at one pole. Some chromatin granules are also found strewn uniformly in its protoplasm while the dark pigment granules are loosely aggregated at the other pole of the organism. These are less in number than the other kind and are apparently the male gametocytes [Plate XXXV, fig. 8, 8 a, and 8 b].

It has not been possible to ascertain any periodicity of fever caused by this protozoan since the animals that came under observation either died or ceased to show the parasite in blood after a day or two. The blood smears made at different intervals from the buffaloes at the Madras Serum Institute showed all the three groups of forms described above and the relative counts of those did not help matters.

## ACKNOWLEDGMENT

Thanks are due to Mr. K. Kylasam Ayyar, Superintendent, Madras Serum Institute, for kindly placing at the author's disposal the blood smears from some of the buffaloes showing the parasite.

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A NEW GORGODERID TREMATODE FROM THE URINARY  
BLADDER OF AN INDIAN MIGRATORY FISH,  
*BELONE STRONGYLURA*

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(Received for publication on 25th March 1938)

(With one text-figure)

BRAUN in 1899 created the genus *Phyllodistomum* (Family—Gorgoderidae Looss, 1902) with *Dist. folium* as the type. Subsequently a large number of species have been described under the genus. Lewis [1935] published a thorough revision of the genus and gave a key to its valid species, described up to 1932. Since then a number of species have been described. Recently, Bhalerao [1937] added two more species to the genus and gave an identification key. In this paper is described a new species of the genus which is parasitic in the urinary bladder of an Indian migratory fish.

*PHYLLODISTOMUM LEWISI*†, n. sp.

Host—*Belone strongylura* V. Hass.

Habitat—Urinary bladder.

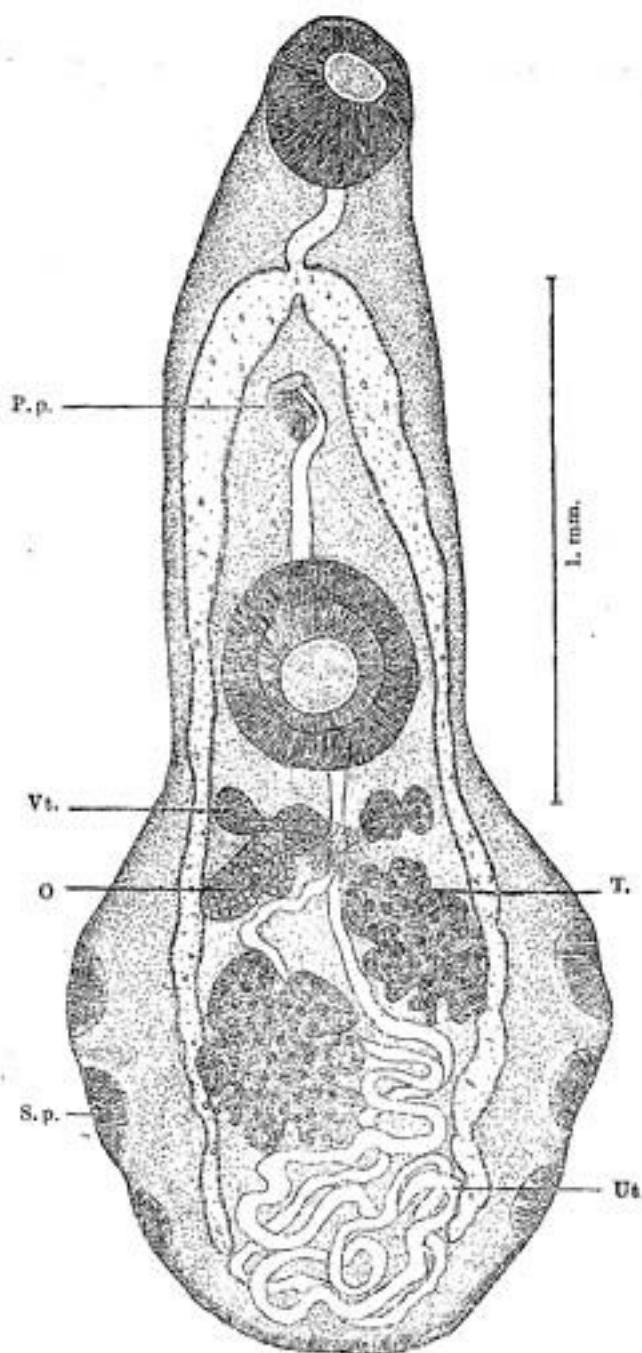
Locality—Allahabad, Ganges and Jumna.

About a dozen specimens of this parasite were collected from the urinary bladder of a migratory fish in the rivers Ganges and Jumna at Allahabad. The trematode has a spatulate body which measures 2·4 by 1·08\* in size and is divisible into a sub-cylindrical, narrow forebody of 1·4 by 0·6 in size, and a broad, foliate hinder body which measures 1·06 in diameter. The body is completely devoid of spines or scales of any kind. The maximum breadth of the body occurs at the level of the posterior testis. On the lateral sides of the hind body are present three pairs of feebly muscular, semi-circular puckerings. The subterminal oral sucker, 0·28 in diameter, opens posteriorly into a 0·2 long, tubular oesophagus which bifurcates into two, straight, simple caeca. The caeca terminate blindly a little in front of the hinder end. The acetabulum measures 0·42 in diameter and is situated at about the middle of the body length.

The testes, two in number, are deeply lobed structures, situated asymmetrically in the intercaecal space in front of the posterior third of the foliate part of the body. The anterior testis, 0·36 by 0·22, lies on the left side. It is separated from the posterior testis, 0·4 by 0·34, by the ascending coil of the uterus. Cirrus sac is absent. A small vesicula seminalis, 0·074 by 0·04, and pars prostatica with few prostate cells are present. A cirrus is absent. The genital pore lies in the median line at about the middle of the fore body.

† The new species is named in honour of F. G. Lewis.

\*All measurements are in mm.

*Phyllodistomum lewisi*, n. sp.

## Key to Lettering.

|       |   |   |   |   |   |   |   |   |   |   |                           |
|-------|---|---|---|---|---|---|---|---|---|---|---------------------------|
| O.    | . | . | . | . | . | . | . | . | . | . | Ovary.                    |
| P. p. | . | . | . | . | . | . | . | . | . | . | Para prestatia.           |
| S. p. | . | . | . | . | . | . | . | . | . | . | Semi-circular puckerings. |
| T.    | . | . | . | . | . | . | . | . | . | . | Testis.                   |
| Ut.   | . | . | . | . | . | . | . | . | . | . | Uterus.                   |
| Vt.   | . | . | . | . | . | . | . | . | . | . | Vitellaria.               |

The ovary has the shape of an inverted dome, with a notch on the top. It measures 0·2 by 0·12 in size and lies obliquely in front of the posterior testis, between the right caecum and the median line. Laurer's canal is present. The shell gland complex lies in the median line in level with the anterior margin of the ovary. The vitellaria are composed of two, transversely elongated, bipartite, lobes situated asymmetrically one on either side of the median line close in front of the ovary. The uterus is intricately coiled in the post-testicular region, but the ascending coil in front of the shell gland mass runs in a more or less straight, median course to open into the small genital atrium. The uterine coils are confined to the intercaecal space and contain a large number of eggs of 0·034 to 0·038 by 0·019 in size.

The excretory bladder is of the type characteristic of the genus.

In having a spatulate body with its posterior portion clearly marked off from the forebody, *P. lewisi*, n. sp. resembles *P. spatula* Odhner, 1902; *P. spatulaeforme* Odhner, 1902; *P. patellare* Sturges, 1897; *P. fausti* Pearse, 1924; *P. superbum* Stafford, 1904; *P. megalorchis* Nybelin, 1926; *P. carolina* Holl, 1929; *P. simili* Nybelin, 1926; *P. unicum* Odhner, 1902; *P. staffordi* Pearse, 1924; *P. lacustri* Lowen, 1929; *P. pearsi* Holl, 1929; *P. angulatum* Linstow, 1907; *P. folium* Olfers, 1816; *P. americanum* Osborn, 1903; *P. hunteri* and *P. lohrenzi* Lowen, 1935. In having ovary posterior to the vitellaria the new species differs from the first four species and resembles the rest. In *P. americanum* the testes are situated in tandem, in the median line near the posterior end. In having the uterus confined to the intercaecal space and the vitellaria composed of lobes, the new species differs from *P. superbum*, *P. megalorchis*, *P. carolina*, *P. simili* and *P. unicum* and resembles the rest. It can be distinguished from the rest of the species by the shape of the body, position of the acetabulum and of gonads and by the size ratio of the suckers. *P. hunteri* and *P. lohrenzi* can be distinguished from *P. lewisi* by the position of the testes. *P. singulare* has a spatulate body with the narrow anterior portion rather sharply set off from the posterior discoidal portion, which has a noticeably crinkled margin. The new species can be distinguished from *P. singulare* by the relative size of the anterior and posterior parts of the body, length of the caeca, position of the vitellaria, extent of the uterus and the absence of the stylet glands.

The author is grateful to the Director and the Pathologist of the Imperial Veterinary Research Institute, Mükteswar-Kumaun for their kind encouragement.

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FIG. 1.

Microphotograph of a preparation of the scrapings showing hyphae and conidiophores showing conidia.  $\times 250$ .

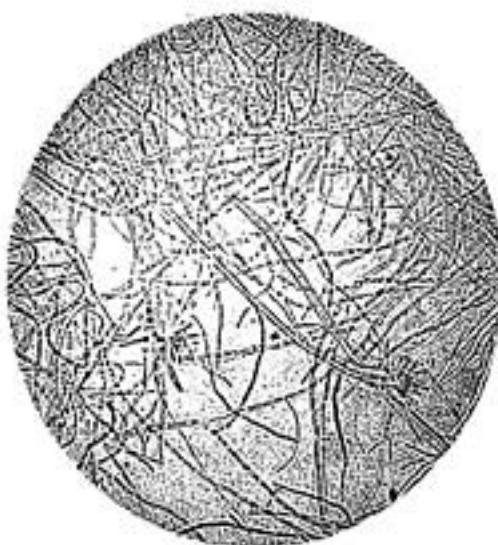


FIG. 2.

Microphotograph of the growth in Sabouraud's medium, showing mycelial network; and Endospores in some free spores, as well as conidiophores bearing conidia.  $\times 250$ .

A PRELIMINARY NOTE ON A SPECIES OF  
*ASPERGILLUS* FROM A CASE OF SKIN  
DISEASE IN A DOG

BY

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(Received for publication on 11th March, 1938)

(With Plate XXXVI.)

In the course of the routine examination of skin scrapings received from the districts, the present case was met with and an opportunity was taken to record this finding since no evidence appears to be available regarding this fungus being the cause of skin disease in dogs.

SUBJECT

A dog of the local breed in Devakotah of Ramnad district (S. India) aged about three years.

HISTORY

This dog was reported to be having a skin affection extending over six weeks, with symptoms of severe pruritus. The disease manifested itself in the form of scabs and scales over the body along with raw and bleeding patches. As a result of its restlessness, it was not feeding well and was rapidly losing condition.

DIAGNOSIS

The material which consisted of crusts from the lesions was boiled in caustic soda solution and the sediment was examined under the low power of a microscope. Felted masses of mycelia were noticed and from these, a few hyphae with conidiophore bearing conidia were found protruding. Under high power, the conidiophore showed a hemispherical base measuring 8 to 20 $\mu$  and from the surface of th's, radiating spores or ascii were seen. (Plate XXXVI, Fig. 1). These spores were either single or in twos and threes united end to end, and the single ones measured 6 to 7  $\mu$  in length. Some of them were round while others pyriform in shape. The hyphae measured 8 to 10 $\mu$  in thickness and varied in length. A very careful examination of the material did not reveal any mange mites or trichophyton. Hence, this affection of the skin is believed to be due to a species of *Aspergillus*.

## CULTURAL CHARACTERS

The material was cultured with some difficulty in plain agar. The technique followed was to tease out from the crusts, small pieces in absolute alcohol and after a couple of changes in the alcohol, plant them in the media. In spite of these precautions, the culture became contaminated in the beginning, but in the course of about four weeks, a fairly good growth of *Aspergillus*, resembling the one seen in scrapings, was obtained with fructifications. It was easy to obtain sub-cultures from this growth and a luxuriant growth was obtained on Sabouraud's medium. In the course of twenty-four to thirty-six hours, a dense white cottony growth was obtained and in another twenty-four hours, the centre of the growth began to change to a light green or bluish green colour with a white border. Later, the whole mass became deep blue in colour and puckered on the surface. In a week to ten days, fructifications were plenty and the colour changed to a honey or brown tint. Examination of a small quantity of the culture in the early stages revealed a dense network of mycelia with stray hyphae and conidiophore bearing conidia. Free spores or ascospores were also found strewn over the area. Some of these mycelia bore endospores in varying stages of development. (Plate XXXVI, Fig. 2.)

## ANIMAL INOCULATION

Attempts to infect laboratory small animals have been futile. Hence this species is probably non-pathogenic to these animals. Experimental transmission to dogs and other animals was not carried out due to various reasons, but it is hoped to do such transmission experiments in the near future.

## DISCUSSION

Regarding the pathological conditions set up by the fungus *Aspergillus*, Buchanan [1922] mentions that the greatest number of cases have been from birds and that the lesions are generally located in the lungs, air sacs and hollow bones. In man and animals, particularly the horse, the same author records this affection in the lungs and air passages with formation of nodules not unlike tuberculosis, leading to occlusion of tubules with the fructification of the fungus and a necrosis of the tissue surrounding the organism. Castellani and Chalmers [1919] report that *Aspergillus* is frequently met with in man causing aspergillosis of various organs such as the lungs, eye, ear, nose, wounds and ulcers, urethra and the skin. The authors quote Montaya with regard to the several varieties of "Pinta" being caused by fungi of the *Aspergillus* genus. Dodge [1936] mentions a large number of species of *Aspergillus* affecting man, birds and other animals, but does not record any skin affection being caused by the fungus. However, he quotes Ballagi and Laubal [1933] who have shown certain species producing scabs and crusts on cutaneous inoculation in the guinea-pig. Omlin [1926] records dermato-aspergillosis

of the horse and Rivolta and Delprato [1926] have reported dermatomycosis of pigeons caused by *Aspergillus glaucus*, but no record is available about the isolation of the fungus from the skin of dogs. Hence, this appears to be the first case of skin disease in a dog in which a species of *Aspergillus* has been isolated and believed to have caused the disease. A complete report on further work done on the subject will be published in due course.

#### ACKNOWLEDGMENTS

The author is indebted to Mr. T. J. Hurley, Principal, Madras Veterinary College for his encouragement, to Rao Sahib M. Anant Narayan Rao, for his instructions and help in this piece of work and to Mr. Viswanatha Pillai, Veterinary Assistant Surgeon, Devakottah for kindly sending me the material. His thanks are also due to the College artist Mr. Doraswamy Mudaliar for the microphotographs appearing in the paper.

#### CONCLUSIONS

A fungus belonging to the *Aspergillus* genus has been suspected to cause skin disease in a dog. This species is found to be non-pathogenic to laboratory animals. (Rabbits, guinea-pig and pigeon.)

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## STUDIES ON THE AMPHISTOMATOUS PARASITES OF INDIAN FOOD-FISHES

PART II—A NEW TREMATODE OF THE GENUS *Gyliauchen* NICOLL  
FROM AN INDIAN MARINE FISH

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(With Plate XXXVII)

NICOLL, in 1915, created the genus *Gyliauchen* for an interesting trematode infesting the intestine of an Australian pilot fish. Goto and Matsudaira in 1918 described *Dissotrema papillatum*, gen. et. sp. nov., from a Japanese fish. But in the following year Goto pointed out the synonymy between his genus and Nicoll's *Gyliauchen*. In 1929, Fukui created the subfamily *Gyliacheninae* containing two species of the type genus, *G. terachodes* and *G. papillatus*. Ozaki [1933] raised the subfamily *Gyliacheninae* to the status of a family. Southwell and Kirchner [1937] placed the genera *Gyliauchen* and *Opistholebes* [Nicoll, 1915], in the subfamily *Opistholebetinae* [Fukui, 1929], along with the genera *Telotrema* [Ozaki, 1933], *Paragyliauchen* and *Cephaloporus* [Yamaguti, 1934]. In this paper the author adds a new species to the genus *Gyliauchen*.

Family.—Paramphistomidae Fischhoeder, 1901.

Subfamily.—Opistholebetinae Fukui, 1929.

Genus.—*Gyliauchen* Nicoll, 1915.

*Gyliauchen ozakii*, n. sp.\*

Host.—*Harpodon nehereus* Ham.

Habitat.—Intestine.

Locality.—Karachi, (Arabian Sea).

This is a very common parasite in the intestine of a marine fish in the Arabian Sea, the frequency of infestation being nearly sixty per cent. The number of specimens in a single host was never found to be more than six.

\* The new species is named in honour of Dr. Y. Ozaki,

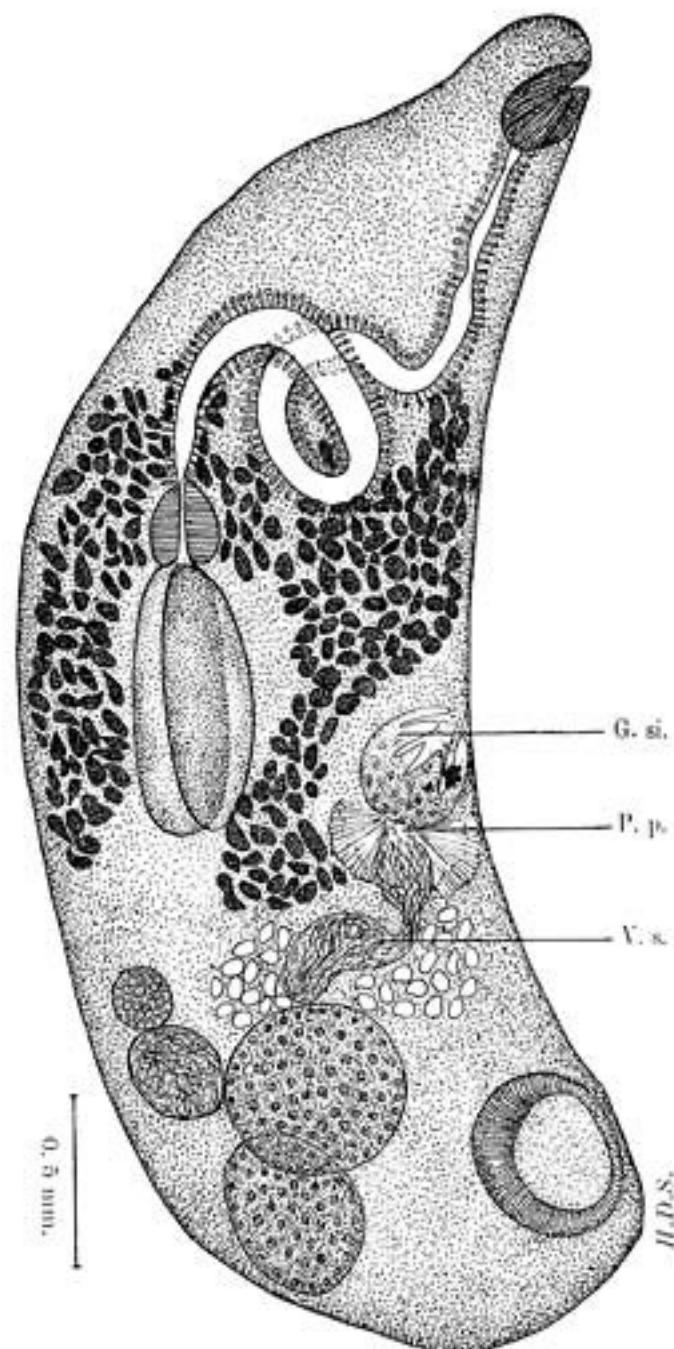
The body is plump, elongated, with convex dorsal and concave ventral surfaces. There are no cuticular spines but cutaneous gland cells are well developed, specially along the sides. In permanent mounts the body measures 2·5 to 4·76\* in length and 0·9 to 1·44 in maximum breadth which occurs at about the middle of the body. The oral sucker is an oval structure of 0·3—0·34×0·18—0·22 in size and opens subterminally on the ventral surface. It is followed by a long, tubular and characteristically coiled oesophagus measuring 1·8 to 3·0 in length and 0·1 to 0·14 in width. It is surrounded by prominent gland cells all along its course. The oesophageal bulb is well developed and measures 0·26—0·28×0·22—0·24 in size. The intestinal caeca are two elongated oval structures of 0·66—0·94×0·4—0·44 in size extending posteriorly almost up to the base of the middle fifth of body length. The acetabulum is situated on the ventral surface a little removed from the posterior end and is of 0·36—0·5 in diameter.

The testes, two in number, are obliquely situated, but at times they are found overlapping each other. The posterior testis, 0·42—0·48×0·38, lies usually in level with the acetabulum. The anterior testis, 0·38—0·54×0·42—0·58, lies obliquely in front of the posterior testis and partly overlapping the latter. The vesicula seminalis is a fairly long, wide tube of 0·5—0·56×0·12—0·18 in size and is divided into two parts by a constriction. It opens anteriorly into a small, swollen, pars prostatica of 0·1—0·16×0·08—0·1 in size, which is surrounded by numerous prominent and closely aggregated prostate gland cells. The ductus ejaculatorius is a 0·1—0·14 broad tube of 0·22—0·3 in length and is lined internally with irregular, cuticular plaques. The genital sinus is a peculiar, spherical, eversible structure of 0·42—0·6×0·3—0·5 in size, with its mouth guarded by several fairly broad, triangular, chitinous processes. The genital pore lies in level with the middle of the intestinal caeca.

The small, spherical ovary of 0·12—0·2 in diameter occupies varying positions in the neighbourhood of the anterior testis. It is usually situated obliquely in front, but may be lateral or partly overlapping the anterior testis occasionally. The receptaculum seminis, 0·34—0·48×0·12—0·26, lies between the ovary, anterior testis and the body wall. A small Laurer's canal is present. The vitellaria consist of a large number of oval follicles arranged in the form of an inverted U, extending from half the distance between the anterior end and the posterior extremity of the intestinal caeca to a little distance beyond the latter. The follicles never overlap the intestinal caeca. The vitelline ducts are as in the type species. The uterus is confined to the space between the testes and the intestinal caeca. Rarely it may extend to the hinder level of the posterior testis. The eggs are numerous and measure 0·076—0·087×0·041—0·049 in size.

Of the two hitherto recorded species of the genus *Gyliauchen*, *G. ozakii*, n. sp. resembles the type species in the general shape of body, topography of the gonads, peculiarities of the digestive system, course of the vitelline ducts

\*All measurements are in mm.



*Gyliamorpha ozukii*, n. sp.

*Key to Lettering*

|        |    |                    |
|--------|----|--------------------|
| G. si. | .. | Genital sinus      |
| P. p.  | .. | Pars prostatice    |
| V. s.  | .. | Vesicula seminalis |



and size of eggs. But it differs in the course of the oesophagus, posterior extent of the ceaca, extent of the vitellaria, absence of the cirrus sac, presence of a peculiar genital sinus and differences in measurements.

The author is deeply grateful to the Director and the Pathologist of the Imperial Veterinary Research Institute, Mukteswar-Kumaun for their kind encouragement.

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## NEW ALLOCREADIIDS (TREMATODA) FROM INDIAN MARINE FOOD-FISHES

PART III—*PEDUNCULACETABULUM PEDICELLATA* N. SP.  
FROM THE GUT OF *CHILOSCYLLIUM INDICUM*

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(Received for publication on 25th March 1938).

(With one text-figure.)

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### *PEDUNCULACETABULUM PEDICELLATA*, N. SP.

Host—*Chiloscyllium indicum*.

Habitat—Intestine.

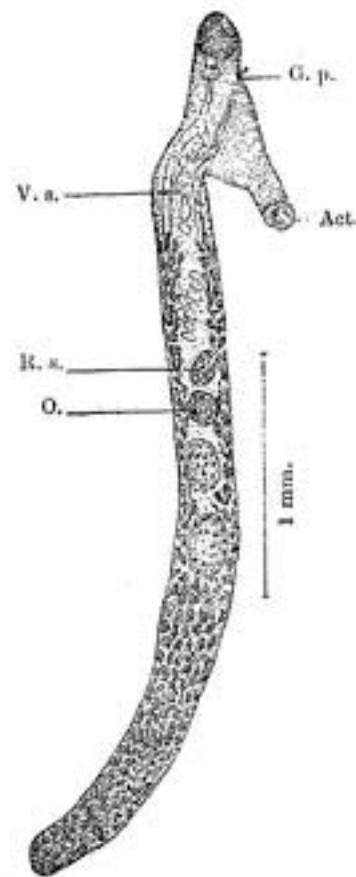
Locality—Puri, Bay of Bengal.

DURING an extensive parasitological examination of marine food-fishes at Puri, the author came across two mature specimens of this parasite. The worm has an elongated narrow body with a nearly uniform breadth. In permanent mount the type specimen measures 3·9\* in length and 0·24 in maximum breadth. The cuticle is studded with extremely minute spines. Minute, deeply staining gland cells are present all over the body. The terminal oral sucker measures 0·16 by 0·18 in size. It is nearly three times the size of the acetabulum which is 0·06 in diameter and is situated on a pedicel 0·48 long. The oral sucker is followed by a small prepharynx, a pharynx and an oesophagus, 0·24 long, which bifurcates into two simple caeca extending to the hinder end.

The two, oval testes are situated one behind the other in the median line at about the middle of body length. The anterior testis of 0·18 by 0·14 in size is slightly smaller than the posterior testis which measures 0·26 by 0·14 in size. The narrow and elongated cirrus sac is enlarged posteriorly and markedly attenuated anteriorly. It extends in a serpentine course from the genital atrium to the beginning of the vitellaria. The genital atrium is situated on the left body margin in level with the pharynx. The cirrus sac encloses an elongated vesicula seminalis, 0·14 by 0·04 in size, pars prostatica, ductus ejaculatorius and a small protrusible cirrus.

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\*All measurements are in mm.

*Pedunculacætabulum pedicellata, n. sp.**Key to Lettering*

|               |                       |
|---------------|-----------------------|
| Act . . . . . | Acetabulum.           |
| G. p. . . . . | Genital pore.         |
| O. . . . .    | Ovary.                |
| R. s. . . . . | Receptaculum seminis. |
| V. s. . . . . | Vesicula seminalis.   |

The small, spherical ovary of 0·1 in diameter lies close in front of the anterior testis. A small receptaculum seminis of 0·14 by 0·1 in size is situated just in front of the ovary. A small Laurer's canal is given off from the duct of the receptaculum seminis. The shell gland complex is diffuse. The vitellaria are composed of numerous, small, oval or pear-shaped follicles, occupying the whole of the post-testicular space and extending laterally almost up to the level of the first quarter of the body length. The uterus is pre-ovarian and intercaecal and contains small, operculate eggs of 0·06–0·07 by 0·04–0·05 in size.

The genus *Pedunculacelabulum* was created by Yamaguti in 1934 and so far contains only the type species—*P. opisthochis*. The new species differs from the type species in the presence of cuticular spines, position of the gonads and the genital pore and the arrangement of the vitellaria in front of the testes.

The author is grateful to the Director and the Pathologist of the Imperial Veterinary Research Institute, Mukteswar-Kumaun for their kind encouragement.

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Yamaguti, S. (1934). *Jap. J. Zool.* 5, No. 3, 249-541.
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## ABSTRACTS

The minimum vitamin-A and carotene requirements of cattle, sheep and swine. H. R. GUILBERT, R. F. MILLER and E. H. HUGHES (1937), *J. of Nutrition*, 13, 543.

THE authors report in this article the results of their investigations on the minimum vitamin-A and carotene requirements of cattle, sheep and swine. Their observations indicate that night blindness is the first detectable symptom of deficiency to appear on the carotene deficient ration, and the last to disappear as the amount of carotene is increased from sub-minimum to minimum levels. The night blindness test supplemented by a check on liver storage either by depletion or by the antimony trichloride test on extracts of the liver tissue was, therefore, used as a criterion of sufficiency.

Evidence has been presented that the daily dose of vitamin-A or carotene that just prevents night blindness, represents a physiological minimum. The minimum carotene requirement for cattle, sheep and swine was found to be 25 to 30 micrograms daily per kg. body weight, an amount in agreement with similar data on the rat. The minimum vitamin-A requirement on the basis of the analysis and the criteria used by these authors, was found to be 6 to 8 micrograms daily per kg. body weight. These figures also appear to agree well with data on rats. Excellent growth occurred at these levels, but liver storage after extended periods was meagre. From the point of view of storage of vitamin-A in the liver, five to ten times the minimal intake has been found adequate. Significant storage accumulates within a few months at such a rate of intake, but in order that the milk should be richer in vitamin-A the intake should be more liberal.

The observations of these authors confirm the view that vitamin-A requirement is directly related to body weight rather than to energy requirement and that the requirements of other species of mammals may be predicted on this basis. Calculations from clinical data indicate that the human requirement cannot be far different from that of the other species studied. A possible limitation of this may, however, exist in the case of carnivores as they receive this factor in their diet partly as preformed vitamin-A. The authors have also pointed out that although the minimum requirement per unit of body weight is the same both for young and old animals, the former are more susceptible to pathological manifestations during vitamin-A privation. Whether or not the apparently greater resistance of older animals to pathological changes in the tissue is due to inherent differences in the tissues or to slow withdrawal of the last vestiges of reserves from adipose tissues is left open for future experimental observations. [N.D.K.]

Carotene (or vitamin-A) deficiency and some common unsoundness in horses. EDWARDS, J. T. (*J. Roy. Army Vety. Cor.*, 9, 1, 1 and *ibid.* 9, 2, 60.)

THIS article, which is well illustrated, contains an excellent review of the literature on vitamin-A deficiencies in animals and the possible relationship of this deficiency and some of the common unsoundnesses, e.g., shivering, stringhalt, partial paraplegia, roaring, impaired vision and sterility in horses, as suggested by Mitchell, has been stressed.

The tissue changes in animals suffering from carotene deficiency are caused by a degeneration in the body cells generally, characterised by keratinization of the epithelium, demyelinization in the nervous tissue, and probably loss in function of cells in other organs such as the liver, spleen and kidney. In the various species the changes are exhibited clinically by manifestations that are differently localized. Thus, for example, in the less severe forms, in the pig and the dog, the most prominent symptoms are ordinarily those of nervous incoordination, in the sheep those of urinary calculi; in the fowl and ox those of xerophthalmia. But in severe cases all symptoms may appear. Sterility is a very common sequel even in mild cases. Symptoms related to changes in the respiratory and alimentary tract are common. All the changes seen in other animals in vitamin-A deficiency arise in fowls almost simultaneously and are described under nutritional roup in fowls. The occurrence of pasty eyes in ducklings illustrates rather typically some of the effects produced upon the offspring when the diet of the mother has been deficient in vitamin-A. It is likely that some of the defects which in ignorance are spoken of as constitutional are probably as much due to the effects of prenatal nutrition as to true heredity, e.g., deficient growth of embryonic intestinal tissue in the developing chick in the absence of vitamin-B<sub>1</sub>, in the mother and the defective development of embryonic nerve tissue as a result of maternal deficiency of vitamin-A. There is the well-known cumulative effect of malnutrition, the effects of which in experimental animals have been found to extend to at least three or four generations.

Hemeralopia or night blindness, which is an inability to see in dim light, is a common early symptom of vitamin-A deficiency in some species, such as human beings, cows and sheep. Its prevalence during the war among army horses, and camels in Persia and rarely mules has been described. It was believed that it was caused through exhaustion of the sensibility of the retina by the glare of the sun. Recent studies have shown that in the absence of vitamin-A, the retina is unable to synthesize a substance called visual purple (rhodopsin) which is bleached by strong light and the absence of which causes this condition. Some cases of night blindness may probably be coupled with a slight degree of optic nerve degeneration in adult cows, a condition from which the animals recover completely after ingestion of adequate vitamin-A. In the congenitally blind calves, however, the degeneration of the optic nerve would have progressed to a stage beyond repair. In such cases the pupils become widely dilated and a rather characteristic greenish, instead of the normal yellowish, colour is thrown from the retina on looking through the cornea with the animal facing the light or better with the help of an ophthalmoscope. Clouding of the cornea appeared in the later stages of the deficiency.

Experiments were carried out upon horses in the Finnish Army which had shown rather wide-spread and typical malformations of the hoof wall that were suspected to be of dietary origin. No greens were supplied to these horses and no vitamin-A could be found in the samples of hay that was fed to them. A marked improvement, in decreasing order of effectiveness, was brought about by supplementary grazing, A. I. V. silage (acidity below pH<sub>4</sub>), and cod-liver oil and a vigorous growth of healthy horn started from the coronary band.

With the exception of yellow maize, cereals are deficient in carotene or vitamin-A. Certain cereals, such as oats, were particularly liable to 'sop up' vitamin-A in the body and cause symptoms to appear. These symptoms were intensified by the addition of ergot to the cereal diet but could be prevented by including in it adequate carotene or vitamin-A. On extending this work to lathyrism in dogs it was observed that the neurotoxin of Akta became effective in the absence of vitamin-A and carotene. Symptoms of poisoning caused by cotton seed meal and certain other similar meals—convulsions, stiffness and blindness—in cows could be prevented by including in the ration sources of adequate vitamin-A.

Failure of reproduction has been reported as a common symptom of vitamin-A deficiency and it has been observed in range cattle even in the milder forms of this deficiency. In the female there is complete failure or delayed occurrence of oestrus, keratinization of the vaginal epithelium and if conception takes place, depending upon the degree to which the uterine epithelium undergoes changes, there is either death of the foetus and resorption, or abortion or prolonged gestation and difficult parturition. Likewise, marked degenerative changes take place in the seminiferous tissue of the testes in the male and these changes take place earlier than in the case of vitamin-E deficiency. It has been observed in Germany that in cattle there was a close relationship between the breeding ratio and the number of days annually spent grazing. It has long been the experience of trainers and breeders that the chances of mares holding to service are increased if service is delayed until there is grazing available.

The deficiency of vitamin-A (anti-infective) lowers individual resistance to bacterial infections, e.g., brucellosis in cattle, and helminthic infestations of the respiratory and intestinal tracts and other parts of the body e.g., Strongylosis in horses. It may be due to the histological changes with the resulting loss of function in the cells of epithelial surfaces through which the bacteria penetrate or in proximity to which the worms reside. Probably the cells of the internal organs—liver, kidney and spleen—are also damaged so that they are unable to suppress the secondary bacteraemia which occurs after invasion.

It has been observed in French Indo-China that, when the ration of horses and mules was changed from normal to one that was deficient in vitamin-A, they lost condition very severely until they suffered from extreme emaciation. A number of these animals developed eye trouble. Substances rich in vitamin-A or its precursor produced a marked and lasting curative effect very quickly but treatment with arsenical preparations produced only a temporary improvement. Cabbage pulp, sterilized cod-liver oil and egg-yolk were tried and the last named substance in 10 c.c. doses injected subcutaneously once daily was found to be the most satisfactory.

A herbivorous animal under natural conditions should carry a reserve in the liver of not less than 1,000 "blue units" or "Moore's units" per grm. In seventy-eight horses examined it was found that the livers of 37 per cent contained less than 100 B. U. per grm.—in some cases merely a trace; 13 per cent less than 200; 28 per cent less than 500, while only 6 per cent contained 1,000 or more. Furthermore the nerve lesions described are somewhat similar to those described in dogs and rabbits fed on a known vitamin-A deficient diet, i.e., myelino degeneration followed by sclerotic changes in the nervous system especially the afferent (sensory) side. These changes in the peripheral nerves were found to occur in patches, the fibres above and below these patches appearing normal. Young animals, particularly showed a wider distribution of these changes in the body. In detailed post mortem examination of horses suffering from shivering, partial paraplegia and various other localized conditions, the pathological findings were the same—widespread bone and joint lesions and disseminated local degeneration of peripheral nerves.

In view of the high proportion of very low reserves of vitamin-A which may be completely depleted during pregnancy or lactation or infectious diseases like influenza and strangles, the very low carotene content of the usual rations fed to horses, e.g., oats, bran and hay, especially if the hay has been badly cured, and the similarity of nerve lesions and clinical symptoms to those produced by vitamin-A deficient diet in pigs and other animals, e.g., impaired vision, extreme neuro-muscular incoordination, spasms (stringhalt action), partial paraplegia, irregularity in the oestrus cycle, abortions, etc., the hypothesis that vitamin-A deficiency is the cause of some of the common unsoundnesses, collectively termed as "Rheumatic disease", in horses is entirely tenable. Symptoms of shivering could be concealed and the horse enabled to pass a veterinary examination if it was placed in a loose-box or a paddock for three weeks or a month and fed well.

The vitamin-A requirement of animals is related to body weight rather than to energy requirement but the requirement of birds is considerably higher than that of mammals. The daily ration of a horse of 1,000 lb. average weight, ought to contain well above 15 mg. of carotene. An adequate supply would be obtained by grazing 10 to 16 ounces of pasture grass daily. The vitamin-A potency of hay is associated with the degree of green colour (chlorophyll). By means of rapid artificial drying in the dark the potency of the fresh grass remained almost unchanged, but on the contrary if exposed to sun and rain for about a week the loss reached 96 per cent. [R. L. K.]

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**Histoire de la Médecine Vétérinaire.** E. LE'CLAINCHE. (Membre de L' Institut Français) Published by Office Du Livre, Toulouse, 1936.

#### Part I.

#### PREFACE

VETERINARY medicine was born with primitive civilization and was long inseparable from that of humans. Honoured in the Greek, Roman and Byzantine Empires, thereafter it vegetated miserably, forgotten and misrepresented for fourteen centuries.

This was due to Christian insistence upon the importance of the soul, an attribute not vouchsafed to animals. When even the human body might be tortured for the good of the soul, it is not surprising that we find them regarding the succouring of animals a sacrilege against the divine art of medicine. In the middle ages, even in the liberal University of Salerno, where the masters dissected animals in the absence of human bodies, the study of animal diseases was regarded as heretical and men were condemned for undertaking it.

Meanwhile, however, in the East, where philosophy dealt only with life and its mysteries, men and animals were deemed to have an identical destiny and consequently human and animal medicine were held in equal honour. Hence, veterinary science, dead among the Christians, continued to develop in the orient, particularly among the Arabs.

When the Arabs conquered large parts of Europe, they profoundly influenced the then prevailing attitude towards animals and retaught Europe much of the Greek veterinary knowledge that it had forgotten, together with several new additions. This marked the veterinary Renaissance, but the movement had little influence until the eighteenth century when the 'philosophers of nature' sought a basis for their systems in the study of anatomy and physiology. Even then the new attitude did not penetrate to the main body of public opinion, and Frederick the Great encountered insuperable opposition when he attempted to open a veterinary school in Berlin, while, when opening the Dresden school, the Duke of Courland was obliged to handle the carcasses himself to overcome the repugnance of the assistants, and the King of England had to issue edicts for the protection of veterinary students, obliged to touch dead animals in the course of their studies, against popular prejudice.

A century, however, of hard work sufficed to free the veterinary art from its earlier obloquy and to allow it to take its place in science and society.

#### ANTIQUITY

Primitive man was a hunter, at first on equal terms with the hunted, but, when, after thousands of years, he discovered how to polish flints for weapons, he secured dominion over the animal world. Thereafter, men living in tribes, were able to settle where they would and cultivate the earth and domesticate animals for food and tillage.

Although we know nothing of animal medicine at this date, it is reasonable to suppose that on the more valuable animals, primitive surgery, such as that practised on man, was used, but in the dawn of mankind, as they have continued to do almost up to our own time, practice and superstition went hand in hand and magic dominated medicine.

In Neolithic times, while Europe was still emerging from the grip of the Ice Age, India was abundantly peopled, owing to its temperate climate and the fertility of the land. The advanced Indus valley civilization, discovered by Sir John Marshall at Harappa and Mohenjo-Daro, has been proved prior to that of Sumer. There is evidence of the use of the bull, sheep, camel, ox and fowl in this period but the horse appears to have been unknown. When the conquering Aryans wiped out this civilization, its culture passed by way of the conquerors to Persia, Greece and Rome.

## ABSTRACTS

The Sumerian civilization left behind it documentary evidence of medical practice, proving that these peoples considered blood, replenished by food, as the life force and the liver the centre of the body (*viz.* the use of animal livers for fortune telling). Water and fire, mixed with religious ritual, were their main therapeutics. Domestic animals constituted the chief criterion of wealth and in them taxes were paid. Horses were uncommon but occasionally used to draw war-chariots.

In the Babylonian civilization again, pastoral life was of predominating importance and we find shepherds figuring in the kings' entourage. Men were taxed according to the number of animals they owned and animals were protected by law, theft of cattle being severely punished, while the sale, hire and pasturing of animals was regulated. Under these circumstances, we expect and find that veterinary knowledge was considered of great importance, but it was still inextricable from human medicine and still mainly theological, only external wounds and disease, such as fractures and diseases of the eye being treated, while most diseases, considered as divine visitations, were relegated to the care of the priest.

From 1900 B.C.—800 A.D., we find the Aryans invading and settling in Northern India. They brought with them a religion based on the Vedas, which were also the principal repository of Hindu medicine. Most of the books deal only with exorcisms and magic conjurations, since disease was regarded in the first place as the work of innumerable demons ('Pisachas' and 'Rakshas') but the Ayurveda also includes treatises on surgery, the treatment of bodily diseases, demonology, child medicine, toxicology and the use of aphrodisiacs. Herbal treatments are detailed, such as the use of the plant 'kustha' against fever, of the plant 'apamargna' against digestive troubles, and of 'arundhati' to re-establish the secretion of milk in cows; while there is a lengthy description of the various methods used for castrating yoke-animals.

In the Laws of Manu, animals and men are equally protected by law and the position of animals is evidenced by the fact that in many Hindu prayers, animals are placed before men, while the Hindu gods were often thought of in animal forms (*e.g.* Indra as the fiery bull—after the death of Vrtra, his greatest deed of heroism was the delivery of the calf retained in Vata's womb).

Buddhism strengthened the eastern veneration for animal life. In the works of 'Susruta' most illustrious of Indian physicians, it is stated that medical assistance was denied to hunters and trappers of animals. Asoka (270-233 B.C.) forbade the killing of animals and established hospitals (precursors of the modern Pinjrapoles) for them as well as for men. The Singhalese kings expected their army doctors to attend men, elephants and horses indiscriminately. All this was not, however, entirely disinterested benevolence, as these ancient kings derived revenue from the taxes on animal sales.

There is a definite body of Hindu veterinary literature, though the largest reputed supply in the hands of the Brahmins has never been allowed to be seen by any save themselves. However in Kaniska's reign, an anonymous doctor collected and edited all the veterinary knowledge of the previous centuries. This book proves veterinary science to have been mainly concerned with religion and ritual but shows that some surgery

and strict rules of hygiene were practised. Palakapya's 'Hastyurveda' dealt with the diseases, treatment and surgery of elephants. Calihotra of Knndahar wrote in Persian on the diseases of elephants and horses. The Asvavaidyaka deals with the anatomy and exterior maladies of horses, and mentions bleeding, blistering and cauterization, as well as the telling of an animal's age by its teeth. In 1838, Mills, the Principal of the Bombay college, discovered a very old medical treatise in Tamil, the work of a 'Rishi' on cattle. This work shows that even in that far-off era, bovine diseases were classified and treated. They undoubtedly knew rinderpest, anthrax, staggers, dysentery, piroplasmosis, meningo-encephalitis and ague. Their treatments recall Arab medicine and doubtless came from a common source. The medicines are less scientific, prescriptions including ground bones, female milk, urine, cattle excreta and feathers.

The history of veterinary medicine in Greek and Roman times is dealt with at length by Léclainche but cannot be more than glanced at in this abstract. The Greeks were the real founders of veterinary medicine. The Age of Hippocrates was perhaps the most brilliant in veterinary history and has certainly influenced veterinary practice in all succeeding centuries. The Greek veterinarians were neither philosophers nor thinkers but attentive and conscientious observers. Most of them also practised as doctors and their methods are similar to those used for man.

The Romans merely collected and annotated the work of the Greeks. They were compilers and disseminators. They established their classifications from books and not from actual observation and it is useless to attempt to identify the diseases they describe. Their work was more complicated and far less rational than that of the Greeks.

The Byzantine successors of the Romans merely stored the Greek and Roman literature during the Dark Ages of Europe and made further compilations.

Rome imposed her civilization so heavily on large parts of Europe that all countries outside her jurisdiction were held to be 'barbarians' but it is untrue to regard all these peoples as savages. The Druids in Britain had a high standard of education and a certain medical knowledge. Rome imported her war dogs from them. Gaul, too, before Caesar's conquest, had a splendid cavalry, a well-developed agricultural system and intelligent methods of cattle breeding. It produced fine wool from selected flocks and exported the best meat in Europe.

*Plagues and contagious diseases of antiquity.*—Epidemics and contagions have played a part in the history of mankind that poets have been prone to exaggerate, while it has not been fully appreciated by historians. For example racial migrations have over and over again been caused by the necessity of avoiding diseases harboured in the soil in certain regions. Epidemics have become more serious as the population of the globe has expanded and its peoples taken to living together in groups. At all times these have caused terrific disturbances in all parts of the world, decisively influencing the existence and destinies of peoples.

Contagious diseases have been known from the earliest times and occasionally isolation of the infected practised, but this only incompletely, and only in certain classes of disease, not, for instance in the case of the rapidly diffused plagues, which were submitted to as visitations of the anger of God.

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For twenty centuries a plague of extreme gravity, recurring at intervals, appeared in the Eastern Mediterranean and spread westwards. It is heard of in mythology and legend, but first definitely described in the Bible (Exodus, Chap. IX) along with many other scourges meted out by Jehovah to the Egyptians to punish them for enslaving the Israelites. It is here described as being characterized by the appearance of ulcers and was responsible for the deaths of enormous numbers of men and animals. Similar plagues are described :—by Ovid in his *Metamorphosis*, as wiping out the population of the Argive Islands in 1295 B.C. and also attacking dogs, birds, cows and wild beasts ; by Homer in the *Iliad* as starting with dogs and proceeding through horses to men ; and by other Greek and Latin writers as having occurred in 735 B.C., 488 B.C., 461 B.C., 451 B.C., 430 B.C., 424 B.C., 397 B.C. and 212 B.C., all as originating in dogs and finally affecting men. In 130 A.D. it is again described more fully as a septicæmia followed quickly by rigors, intense fever, sanguous expectorations, coughing, unconsciousness and death. This plague does not correspond to any known form and may now happily be extinct.

Epizootics peculiar to animals have also been described by these ancient writers, among them some recognisable as pleuro-pneumonia in bulls, influenza of horses, sheep pox and rabies of the dog ; others are less easy to identify and it may be that they have since disappeared or been profoundly changed in the course of centuries. Rinderpest is heard of for the first time in 370 A.D. (identified by Serverus Sanctus) and reported to have come from the East and devastated Belgium, Flanders and finally Rome. Rabies, described by its transmission through dog bite to man, is frequently mentioned. Aristotle recognised it also in horses, bulls and foxes. Apuleius mentions that the sufferers will not touch water. Philomenos (3rd century) advocated cauterization of the bites as the most effective treatment. Glanders and pleuro-pneumonia were definitely differentiated by the Greeks but forgotten again by the Romans. Columella describes all herd diseases by the generic name of 'plague' while Vegetius uses the term 'maleus' for all contagious diseases ending in death. He recognised eight forms of maleus, which appear to have included all known contagions but which, except for rabies, are unidentifiable. The veterinarian 'Chiron' recognised under the term 'maleus' diseases which correspond to human plague, bull 'verago', sheep pox, rabies and 'aceus' in pigs.

*Diseases of horses.*—Classical knowledge of horse diseases is to be learnt mainly from the *Hippiatrica*, a Byzantine compilation of earlier writers, many of whose works are now lost. It is remarkable to discover that nearly all affections of the bodily organs classified to-day were then known and possessed a very similar nomenclature.

Digestive maladies are dealt with at length and include dental troubles such as abscesses and decay, tumours of the palate and lammas. The most common treatment was by bleeding and it is evident that they possessed an adequate knowledge of how to avoid the arteries. Apsyrtus describes lancing of abscesses as dangerous. Colics are differentiated from abdominal troubles by their causes and afterwards by their lesions. They are held to follow over-eating, alterations in fodder, drinking of bad water or the presence of worms in the intestines. Their descriptions of the lesions are confused but one can recognise intestinal congestion by the symptoms indicated, that

is violent pains, rolling on the ground, groaning, sweating and sudden collapse. Distension of the stomach by gas is given the picturesque name of "the drum". Intestinal invagination, volvulus, serious diarrhoeas, dropsy and tympanites are also clearly described. They were treated with douches, scarifications and punctures to allow the introduction of a reed to withdraw fluid.

Liver complaints received extensive study but those described are difficult to identify and may be peculiar to certain regions, due to microbial or parasitical invasions or chronic toxæmia from poisonous plants.

Infections of the respiratory tracts are only studied in their outward manifestations. Polypus of the nose is declared frequent in horses and treated by excision or cauterization. The description of pneumonia is confused and often clearly includes glanders. It was treated with fumigations, purgatives, bleeding and strange and complex medicines.

Only those urinary troubles easy to investigate were studied. They differentiated between dysuria, strangury and ischuria. The treatments were numerous and not very effective, for example massage of the loins or of the full bladder through the rectum, or the introduction of irritating substances such as salt into the urethra.

Affections of the central nervous system were studied only in their manifestations, such as the phenomena of excitability, frenzy, epilepsy, contraction, tetanus and paralysis. Tetanus was confounded with exposure, a state frequent in army horses during mountain passages in winter. It was treated by hot baths and bleeding. One form of paraplegia, described as characterised by staggering and the emission of thick, black urine, evidently corresponds with enzootic haemoglobinuria. Paralysis of the penis, inguinal hernia frequent in old horses and vaginal-uterine prolapse are all described and their treatment detailed.

Diseases of the eyes are often mentioned and appear to have been very frequent in Asia and Africa, particularly endemic ophthalmia, conjunctivitis, ulcers of the cornea and staphylooma. They operated upon cataract.

Dislocations, fractures and bruises were minutely studied but major fractures were regarded as incurable. A considerable amount was known regarding ligamentary and tendinous lesions but little of articular arthritis and "founder". Deep hoof wounds were cut and cauterized but superficial ones were left to the care of the owner, who cleaned them with copper sulphate. These were very common, as among the Greeks shoeing was unknown and among the Romans, metal shoes often of silver or gold, tied by bandages to the hoof, were mainly used only for show. The ancients relied chiefly on keeping the stable floors, made preferably of wood, dry and clean, to avoid serious foot troubles.

*Diseases of cattle.*—These did not receive the same attention as those of horses, especially among the Greeks, who considered cattle of little importance. There were, however, various writers who dealt with them, Elein in the third century B.C., Epicarmus, the "spiritual father of Pliny and Columella," in the second century B.C. and Praxamus in the first century A.D. A Byzantine compilation called the 'Geoponica' prepared by Magnon of Carthage, sums up all the agricultural knowledge that

preceded his time, and it includes texts on the pathological conditions of ruminants and pigs. It is clear that at this date cattle were considered mainly from their importance in agriculture and economics; but Magnon quotes Democritus as saying, "the causes of nearly all diseases of cattle are unknown".

Careful rules of hygiene were observed but treatments were mostly unscientific, such as treating hoof trouble with fermented urine. Powdered snake skin was much used in prescriptions. The principal causes of disease were held to be excesses of heat, cold or labour. Fowl and pig excreta were considered very dangerous. A few parasitical invasions were recognised, particularly fluke disease, which caused enormous losses in herds during wet years. External diseases are more adequately dealt with, but more summarily than in horses. Dislocations due to methods of stabling were frequent.

*Practice of veterinary medicine in antiquity.*—There is definite evidence of the existence of large numbers of professional animal doctors in early times, particularly among the Egyptians and Sumerians, though at first their duties were mainly surgical, in which branch they were always more adept than in medicine. Among the Greeks and Romans, veterinarians were indispensable, being attached to the army cavalry and transport services, or stationed at the various mail relay posts, they were also in charge of the beasts destined for the arena and the medical care of domestic animals. They were always included in the various Emperors' suites and took sixth place among the twenty-eight technicians attached to the legions.

#### THE MIDDLE AGES

After the fall of the Roman Empire, much of her old culture remained alive in Byzantium but some was taken over by her barbarian conquerors themselves. For instance the Anglo-Saxon kings in England founded three medical schools on Roman lines and patronized agriculture and cattle breeding, while the Franks bred cows, sheep and goats and above all the large black pig, and undoubtedly practised a little animal medicine.

By the ninth century, however, fresh waves of barbarian invasions had plunged Europe in further warfare and the feudal system subjected the whole continent to incessant fighting, pillage, famine and disease, with disastrous effects upon culture.

In Byzantium alone, the study of medicine was followed and even there was so subordinated to Christian dogma that it gradually decayed. The triumph of Christianity in the West meant that for centuries freedom of thought and scientific investigation were punishable as heresy. Even the medical knowledge of the Greeks was abhorred because it was pagan. Rational medicine ceased to be practised and mankind resigned itself to disease, as to other calamities, without evoking other help than the Will of God. Religious medicine, which we have seen in all ages co-existing with scientific medicine, was in this period substituted for it. Much has been written on the mystical medicine of the Middle Ages, which appears to have consisted mainly of invocations to particular saints. There were special saints (e.g. St. Antony, St. George, St. Nicholas) who were supposed to interest themselves particularly in the protection of animals.

Not until the time of the Crusades do we find any amelioration of this condition. When the Crusaders' horses were decimated by glanders and the crusading army spread all kinds of disease in their wake, they were forced to take some measures for the protection of their animals but these consisted mostly of the administration of drugs and petty surgery and the theory was confined to camp-fire discussions. Nothing was written at the time and much of the practice must have been illogical and barbaric; though the social position of the veterinarian was undoubtedly improved, when even the nobility deigned to interest themselves in and acquire some knowledge of veterinary practice.

Not, however, till the time of the Arab invasions of Europe, was there any real return of scientific knowledge to Europe. The Arabs exercised a profound and beneficial influence on medicine, not only because of their very real love for and interest in their horses, but because they rediscovered the old Greek knowledge in Byzantium and returned it to Europe. Islam was for many centuries the home of civilization. The Abassid Caliphs were patrons of science and art, and encouraged the study of Greek, Syrian, Hindu and Persian texts. There were also original writers, the two greatest probably being Abouzascaria and Abour-Bekr, whose book is, even to-day, described as a 'monument to the glory of the horse'. Another writer, the much discussed 'Ippocras the Hindu' a native of Sind, was attached to the court of Chosroes the Great of Persia. Apart, however, from returning the ancient learning to Europe and adding a little to the knowledge of horse breeding and surgery and enriching pharmacy, they added little to the sum of knowledge and nothing at all to that regarding cattle medicine.

*Plagues and contagions in the Middle Ages.*—The Middle Ages suffered incessantly from plagues and contagious diseases of all sorts. Here are a few indications to give an idea of their frequency and gravity in all countries.

The sixth century saw the return of the plague of animals and men so often described in the ancient world. It desolated Gaul. During the same century rinderpest ravaged Central and Western Europe and there were further grave outbreaks in A.D. 569-679, 850, 870, 878, 886, 940-42, 1170-72, and from 1223-25. Nearly all bovine contagions appear to have come from the East and though not of long duration, destroyed enormous numbers.

In 791 A.D., all the horses in Charlemagne's army during his war against the Huns were stricken with glanders and so many died that he was obliged to interrupt his campaign. In 801 his whole empire was ravaged by the plague which killed both men and beasts. Here we meet one of the frequent mentions of 'germ warfare', with the story of an enemy's sending men out with enchanted powders to spread in cattle pastures. In 820-24, the great rains and floods caused a terrible sickness which devastated the Frankish Empire.

Eight hundred and forty, 992 and 1130 A.D. are described as 'periods of gloom, horror and calamities of all kinds'. They saw twenty deadly epidemics which attacked men only, six which attacked cattle, two which attacked horses, twelve which spread through all the animal world and by four of which men and animals were equally affected.

In 1275, sheep pox was imported into England from France and the magnificent flocks bred by the Cistercian monks were for twenty-eight years decimated or completely destroyed in all parts of the kingdom, while England's woollen trade was seriously affected.

In 1301 and 1313, equine influenza had serious hold in Rome. During 1345-50 owing to the Black death (small pox) men, horses, cattle, sheep and goats died in millions.

*The Renaissance.*—By the fifteenth century the Renaissance had hold of Europe and a critical spirit was abroad. The works of the Greeks and Romans including the Hippocratic and the Geponica were being re-edited.

Among the most potent influences responsible for this re-awakening in the veterinary world, was the work of the Arabs in Sicily and Spain. Roger II of Sicily (d. 1154) was a great patron of the arts and sciences. At his instigation Moses of Palermo translated into Latin the writings of Hippocrates the Hindu. He also prohibited the practice of medicine by the unauthorized. His work was continued by Frederick II of Naples and Sicily. He established the great medical school at Salerno and drew up for it a model constitution and programme of studies. These were to last for five years including active *post mortem* work, together with one year's apprenticeship to a qualified practitioner. He himself studied zoology and anatomy and collaborated with his Master of the Stables, the famous Jordanus Ruffus, in writing a book on farriery, "Mareschalerie". This book promised a new era in veterinary medicine and shows its author to have been a faithful observer of nature, who wrote from personal experience and was free from the superstition and scholasticism of previous writers. For the first time we meet a scientific classification of diseases. His work was translated into Latin, Italian, German and Hebrew.

The Arab influence penetrated to Italy where numerous qualified practitioners were employed and other veterinary writings undertaken. Paracelsus, Leonardo da Vinci, Ruini, Rusius, Maurus and Aldrovandi all produced original veterinary works, while others such as Gessner and Jean Revel were busy translating the classics.

Nevertheless this promise of a new era of veterinary medicine was never fulfilled. The writings of Ruffus and the others were soon forgotten or distorted out of recognition.

Why, it may be asked, did veterinary medicine benefit so little from the Renaissance? There are two main reasons. Firstly, theology still reigned supreme in Europe and the Renaissance did not change men's ideas regarding the soul. Europe still suppressed such freedom of thought and expression as had been allowed by the Sicilian kings. Secondly, the veterinary profession could at that time only have progressed, if the medical profession had been willing to offer it a helping hand. It was not, but on the contrary opposed animal medicine with disdainful hostility. Doctors, who showed an interest in animals, were ostracised. For example Herodotus was condemned by his colleagues for publishing an anatomy of the horse on the order of Charles IX. Although for so long the medical profession had recourse to animals for their study of anatomy, it was not for centuries that the idea of comparative anatomy really developed. [J. F. S.]

**A further contribution to the proposal of a new classification of Trypanosomes.**

I. JOCONO. (*The J. of Trop. Med. and Hyg.* (1938), 41, 53-57, with 14 figs.)

In this article the author has brought forward further evidence in support of his previous publication [ Jocono, 1935. *Annali di Med. Nav. e Coloniale*, i, fasc. 1-11 ] in which it was proposed that the genus *Trypanosoma* should, provisionally, be represented by *Trypanosoma rotatorium* of frog, while the other haemoflagellates of man and animal should be regrouped under the genus *Castellanella*. Development of *T. rotatorium* has been observed *in vitro* and a new medium has been suggested which consists of the following ingredients :—

|                                     |   |   |   |   |   |   |   |   |   |           |
|-------------------------------------|---|---|---|---|---|---|---|---|---|-----------|
| Distilled water                     | . | . | . | . | . | . | . | . | . | 150 c.c.  |
| Peptone                             | . | . | . | . | . | . | . | . | . | 0·65 gm.  |
| Glucose                             | . | . | . | . | . | . | . | . | . | 0·30 "    |
| Sodium chloride                     | . | . | . | . | . | . | . | . | . | 0·10 "    |
| Potassium chloride                  | . | . | . | . | . | . | . | . | . | 0·03 "    |
| Monopotassium chloride              | . | . | . | . | . | . | . | . | . | 0·30 "    |
| Brilliant cresyl blue (1 : 100,000) | . | . | . | . | . | . | . | . | . | 10 drops. |

Under aseptic conditions 0·01 c.c. of blood from the aseccular vein of frog was aspirated by means of Pasteur pipette and mixed with 0·1 c.c. of the culture medium placed previously on a clean slide and covered with a coverslip. Both the slide and coverslip should be sterilized with dry heat at 180°C. Edges of the cover glass are then sealed with melted hard paraffin. In this medium phenomenon of lysis occurred after three days and the process of development showed no further modification in shape or size, contrary to what occurred *in vivo* [ Jocono, 1935, *ibid* ]. Dividing forms were seen by the author at frequent intervals and it was found a parasite divided into two or, at most into four elements. Daughter individuals thus formed gradually elongate and ultimately develop flagellum and rudimentary undulating membrane. The author has also dealt somewhat in detail, with the difference in regard to the protoplasm, nucleus, and kinetosome and basal body as observed in the genera *Trypanosoma* and *Castellanella*, respectively. On the basis of these observations two genera are defined as follows :—

"(1) Genus *Trypanosoma* Gruby, 1843, Haemoflagellates belonging to the family Trypanosomidae and characterised in the mature form by an oval or globular shape (The transverse diameter being only slightly smaller than the longitudinal diameter), a centrally placed nucleus which is small in proportion to the diameter of the parasite and with diffuse chromatin; the parasite with few vacuoles and lacking a basal granule, characterised principally by the location of the kinetosome near and behind the nucleus."

"(2) Genus *Castellanella* Chalmers, 1918, emend. Jocono, 1935. Haemoflagellates of the family Trypanosomidae and in the mature form are typically elongate (the length being considerably greater than the width) with granular or homogeneous cytoplasm and with a granular nucleus which is large in proportion to the diameter of the parasite and located centrally or placed at the aflagellar extremity". [H. N. R.]

Nine years' clinical experience of the Laidlaw-Dunkin method of immunisation against Canine Distemper. WILKINSON, D. E. (1937), *Vet. J.* 93, 256-261.

The author has successfully immunised during a period of nine years about one thousand dogs by the reputed Laidlaw-Dunkin method against canine distemper and has thus given his closest co-operation to Laidlaw, Dunkin and Dalling.

The historic development of the original vaccine-virus method of immunisation into the present day virus-serum inoculation is extensively reviewed. A dose of ferret passaged virus followed within a few hours, by an adequate injection of the specific protective serum constitutes the Laidlaw-Dunkin method.

The confirmatory evidence of active immunity resulting from the inoculations together with its durability has been assessed by authentic and intensive kennel experiments which had involved deliberate and prolonged exposure to the disease by close contact-infection. An instance of satisfactory immunity lasting for even nine years following the immunisation in a bull terrier bitch is cited and has been evidenced by her constant contact with a distemper patient.

Most of the cases where failure of protection was reported, are alleged to be due to faulty technique or mistaken diagnosis; distemper in the dog is seldom easy to diagnose even by experts. The period of incubation which is four days is the first criterion in diagnosis. The author suggests the application of curative value of specific serum to the so-called cases of breakdown of immunity or experimental contact infectivity test of susceptible puppies as aids in the differential diagnosis. A mucoid diarrhoea in the early stages of distemper and not the catarrhal discharges indicative of other infections is a valuable diagnostic aid.

Occasional formation of cysts following the injection of ferret virus, the protein of which is foreign to the dog, is preventable by injecting the virus intradermally. The author discourages the use of cheap substitutes in place of the virus and serum elaborated in Laidlaw-Dunkin method.

His experience in canine practice with particular reference to Laidlaw-Dunkin method of immunisation against distemper warrants its extensive application as a safe, efficacious and reliable measure. [S. G.]

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